7-3-2007

Regulation of Meiotic Cohesion and Chromosome Core Morphogenesis during Pachytene in Drosophila Oocytes

Radhika S. Khetani
Dartmouth College

Sharon E. Bickel
Dartmouth College

Regulation of meiotic cohesion and chromosome core morphogenesis during pachytene in *Drosophila* oocytes

Radhika S. Khetani and Sharon E. Bickel*

Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

*Author for correspondence (e-mail: sharon.e.bickel@dartmouth.edu)

Accepted 3 July 2007
Journal of Cell Science 120, 3123-3137 Published by The Company of Biologists 2007
doi:10.1242/jcs.009977

Summary

During meiosis, cohesion between sister chromatids is required for normal levels of homologous recombination, maintenance of chiasmata and accurate chromosome segregation during both divisions. In *Drosophila*, null mutations in the *ord* gene abolish meiotic cohesion, although how ORD protein promotes cohesion has remained elusive. We show that SMC subunits of the cohesin complex colocalize with ORD at centromeres of ovarian germ-line cells. In addition, cohesin SMCs and ORD are visible along the length of meiotic chromosomes during pachytene and remain associated with chromosome cores following DNase I digestion. In flies lacking ORD activity, cohesin SMCs fail to accumulate at oocyte centromeres. Although SMC1 and SMC3 localization along chromosome cores appears normal during early pachytene in *ord* mutant oocytes, the cores disassemble as meiosis progresses. These data suggest that cohesin loading and/or accumulation at centromeres versus arms is under differential control during *Drosophila* meiosis. Our experiments also reveal that the α-kleisin C(2)M is required for the assembly of chromosome cores during pachytene but is not involved in recruitment of cohesin SMCs to the centromeres. We present a model for how chromosome cores are assembled during *Drosophila* meiosis and the role of ORD in meiotic cohesion, chromosome core maintenance and homologous recombination.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/120/17/3123/DC1

Key words: Meiosis, Cohesin, Synaptonemal complex, Recombination

Introduction

Accurate segregation of chromosomes during meiosis relies on a number of dynamic changes in chromosome morphology that take place within the context of sister-chromatid cohesion. Meiotic cohesion is not only required for the correct segregation of sisters during the second meiotic division, but also ensures that recombinant homologous chromosomes remain physically associated until anaphase I (Bickel et al., 2002; Buonomo et al., 2003; Hodges et al., 2005; Siomos et al., 2001). In addition, arm and centromeric cohesion must be regulated differently during meiosis. When the release of arm cohesion during meiosis I allows the segregation of homologues, centromeric cohesion must be protected and remain intact until anaphase II when sisters segregate to opposite poles (Kerrebrock et al., 1992; Kerrebrock et al., 1995; McGuinness et al., 2005; Wang and Dai, 2005; Watanabe and Kitajima, 2005).

Cohesion between meiotic sister chromatids plays an essential role in assembly of the synaptonemal complex (SC), a tripartite proteinaceous structure that forms between homologous chromosomes during prophase I (Cai et al., 2003; Klein et al., 1999; Pasierbek et al., 2001; Webber et al., 2004). During early prophase I, each pair of sister chromatids undergoes shortening along their longitudinal axes, resulting in the formation of 'chromosome cores' upon which the axial/lateral elements (AEs/LEs) of the SC assemble (Revenkova and Jessberger, 2006; Stack and Anderson, 2001). During pachytene, SC central element proteins join each set of homologous AEs/LEs along their entire length resulting in synapsis of homologues. In many species (yeast, mice, Arabidopsis), meiotic double-strand breaks (DSBs) are essential for homologue synapsis (Page and Hawley, 2003); however, chromosome core formation (axial shortening) does not depend on DSBs (Bhuiyan and Schmekel, 2004; James et al., 2002; Romanienko and Camerini-Otero, 2000). In addition, mutants that lack AE/LE components can still build chromosome cores (Couteau et al., 2004; Pelttari et al., 2001).

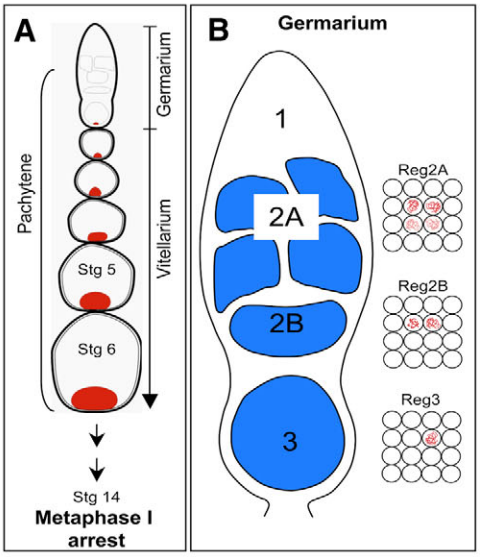
Crossovers between homologous chromosomes, in conjunction with sister chromatid cohesion, are essential for correct chromosome segregation during meiosis I. In most organisms, recombination between homologues takes place in the context of the SC (Page and Hawley, 2003). Although EM studies indicate that the ultrastructure of the SC is highly conserved, SC components in different organisms show surprisingly little sequence homology (Page and Hawley, 2003).

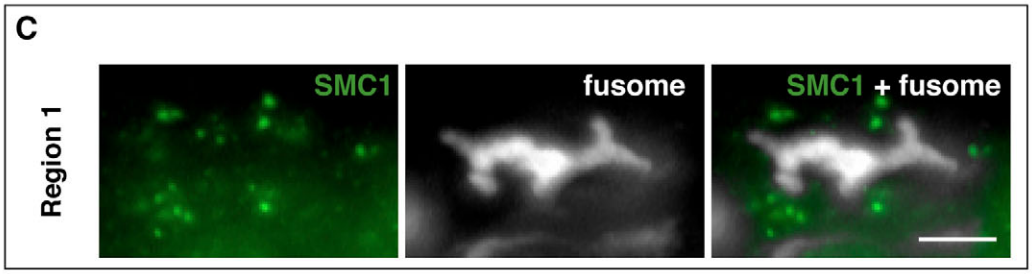
During both mitosis and meiosis, sister-chromatid cohesion is mediated by an evolutionarily conserved protein complex called cohesin that contains two SMC (structural maintenance of chromosomes) and two non-SMC subunits (Lee and Orr-Weaver, 2001; Petronczki et al., 2003). The α-kleisin subunit (Scc1/Mcd1/Rad21) bridges the two head domains of the SMC1-SMC3 dimer and thereby forms a ring that entraps DNA (Nasmyth, 2002; Shintomi and Hirano, 2007). Several meiosis-

specific cohesin subunits have been identified, including the α-kleisin Rec8, which has been shown to be crucial for meiotic cohesion and SC formation in all organisms examined (Klein et al., 1999; Molnar et al., 1995; Pasierbek et al., 2001; Petronczki et al., 2003).

In *Drosophila*, four cohesin subunits have been uncovered through sequence analysis (Adams et al., 2000; Hong and Genetzky, 1996; Warren et al., 2000a) and the localization and function of mitotic cohesin has been examined in *Drosophila* embryos and tissue culture cells (Valdeolmillos et al., 2004; Vass et al., 2003; Warren et al., 2000a; Warren et al., 2000b). However, little is known about the localization and dynamics of the cohesin complex during *Drosophila* meiosis.

Drosophila oogenesis is an excellent system to study meiosis, as each *Drosophila* ovary is composed of approximately 10-30 ovarioles (Fig. 1A) that contain a linear array of oocytes at progressive developmental stages from mitotic germ-line stem cells to metaphase-I-arrested oocytes (King, 1970). Meiosis initiates in the germarium, the most anterior structure of each ovariole (see Fig. 1A,B). Germ-line stem cells in region 1 of the germarium (Fig. 1B) undergo four rounds of synchronous mitotic divisions resulting in 16 interconnected cells that comprise a 'cyst' (Spradling, 1993). As cysts mature, they move toward the posterior end of the ovariole. All germ cells within a 16-cell cyst undergo pre-meiotic S phase synchronously and prophase I of meiosis initiates in germarial region 2A where up to four cells per cyst initiate SC assembly (Fig. 1B). In addition, meiotic DSBs are induced in region 2A (Jang et al., 2003; Mehrotra and McKim, 2006), but unlike several other organisms, synapsis in *Drosophila* does not depend on DSBs (McKim and Hayashi-Hagihara, 1998). As each cyst moves through the germarium,





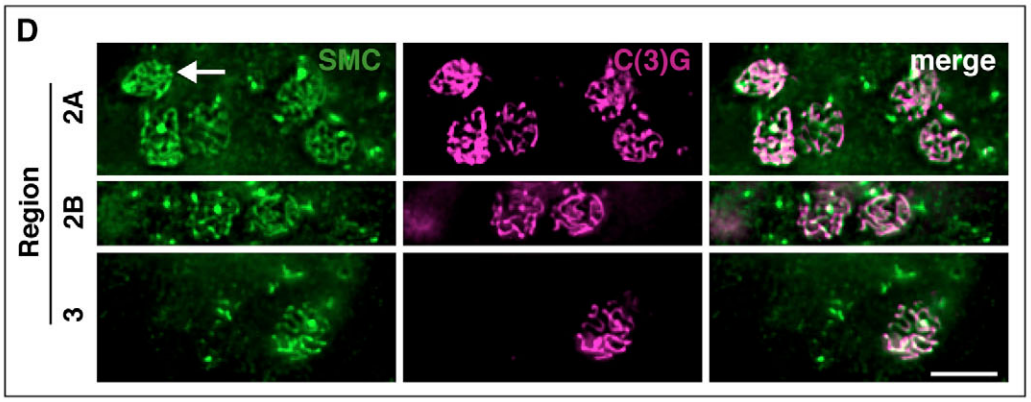


Fig. 1. Cohesin SMC localization during early oogenesis. (A) Diagram of a single ovariole with the youngest stage at the top. Each ovariole contains 'cysts' composed of 16 interconnected germ cells, one of which is the oocyte (red). Meiosis initiates at the anterior tip of the ovariole in the germarium. The remainder of the ovariole is called the vitellarium. As cysts progress through oogenesis, they move toward the posterior end of the ovariole. In stage 14, the oldest egg chamber in an ovariole, the oocyte is arrested at metaphase I. Passage through the oviduct triggers the resumption of the meiotic divisions. (B) The germarium is made up of four regions: region 1, region 2A, region 2B and region 3 at the posterior end. Individual cysts are depicted in blue. On the far right is a diagram showing the assembly of SC (red) in a subset of cells within region 2A cysts. As cysts mature and move to the posterior end through the germarium, the SC becomes restricted to the ooctye. (C) Bright foci as well as diffuse SMC1 signal (green) is visible within region 1. The fusome localization pattern (white) suggests this is either an 8-cell cyst or an early 16-cell cyst. Bar, 4 μm. (D) Simultaneous staining with antibodies against SMC1 and SMC3 shows localization of cohesin SMCs (green) coincident with the SC protein C(3)G (magenta) in different regions of the germarium. In region 2A, two cysts are visible, with two to three cells per cyst containing thread-like SMC1/3 signal (arrow). In region 2B, SMC1/3 threads are restricted to two nuclei per cyst, and by region 3 long stretches of SMC1/3 signal are visible only within the oocyte. Bar, 5 μm. All panels represent projections of deconvolved *Z*-series using whole-mount germaria.

the SC breaks down in all but one nucleus so that, by region 3, full-length SC is restricted to the oocyte, which lies at the posterior end of the rounded cyst (Fig. 1B). As cysts continue to grow and mature, they leave the germarium and move into the 'vitellarium' (King et al., 1956). The oocyte remains in pachytene with full-length SC until vitellarial stage 6 (Carpenter, 1975; Page and Hawley, 2001); however, the remaining 15 cells within each cyst adopt a nurse cell fate and enter an endo cell cycle, during which multiple rounds of S phase in the absence of intervening M phase results in polyploid cells (Dej and Spradling, 1999).

Meiotic cohesion in *Drosophila* depends on the novel protein, Orientation Disruptor (ORD) (Bickel et al., 1996; Bickel et al., 1997; Mason, 1976; Miyazaki and Orr-Weaver, 1992). In mutants lacking ORD function, sister-chromatids segregate randomly through both meiotic divisions, consistent with complete absence of meiotic cohesion (Bickel et al., 1997). In addition, homologous recombination is severely reduced in ord^{null} females and SC assembly and maintenance are disrupted (Bickel et al., 1997; Webber et al., 2004). Immunolocalization studies have demonstrated that ORD is enriched at the centromeres of meiotic chromosomes in both males and females (Balicky et al., 2002; Webber et al., 2004). In addition, ORD localization along the arms of female meiotic chromosomes coincides with that of the SC protein, C(3)G (Webber et al., 2004).

Here, we investigate the localization and dynamics of two cohesin subunits (dSMC1 and Cap/dSMC3) during early prophase I in *Drosophila* oogenesis. SMC1 and SMC3 localize along the arms and are enriched at the centromeres of all 16 cells within each germ-line cyst. In nuclei that build SC, cohesin subunits coalesce into chromosome cores that provide the scaffold for SC assembly. We find that formation of chromosome cores depends on the α-kleisin C(2)M, and that the cohesion protein ORD is essential for cohesin loading at centromeres and for maintenance of chromosome cores. Our data support the argument that during meiosis, the establishment of centromeric cohesion is regulated differently than on the arms. Moreover, our results provide insight into the interconnected roles of meiotic cohesion, chromosome cores and homologous recombination.

Results

Accumulation of cohesin SMCs on chromosomes of pre-meiotic cells

To analyze the behavior of cohesin subunits during meiotic progression in the *Drosophila* ovary, we generated antibodies against *Drosophila* SMC1 and SMC3 peptides. Following affinity-purification, SMC1 and SMC3 antibodies each recognize a single predominant band at the predicted molecular mass in embryo extracts and a doublet/triplet in ovary extracts (see supplementary material Fig. S1a). In addition, when germ-line clones are generated that are homozygous for the *smc1* excision allele, $smc1^{exc46}$ (Dorsett et al., 2005), no SMC1 signal above background is observed (see supplementary material Fig. S1b). Because affinity-purified SMC1 and SMC3 antibodies display very similar staining patterns in *Drosophila* ovaries (see Fig. 6), they were combined for most of the experiments described below to maximize signal intensity (here referred to as SMC1/3 or SMC).

Fixation and staining of intact ovarioles (whole-mount preparations) revealed several distinct cohesin SMC staining patterns within the germaria of wild-type females; multiple regions of each germarium contained bright foci, as well as diffuse staining and nuclei with a thread-like SMC signal (see supplementary material Fig. S2). Although we do not detect cohesin SMC staining in germarial stem cells and early cystoblasts in region 1 (see supplementary material Fig. S2, asterisk), bright foci as well as diffuse SMC localization are visible in the nuclei of germ-line cysts within region 1 (Fig. 1C). The bright SMC foci correspond to centromeres as confirmed by co-staining with CID (see below, Fig. 4), a centromere-specific histone H3 variant (Blower and Karpen, 2001; Henikoff et al., 2000). The diffuse staining in pre-meiotic cells most probably corresponds to cohesin localization along chromosome arms. This same localization pattern has been observed for the cohesion protein ORD in germ-line mitotic cysts (Webber et al., 2004).

Thread-like cohesin SMC signal coincides with the SC in pachytene nuclei

As 16-cell cysts enter region 2A of the germarium, up to four nuclei in each cyst begin to assemble a SC and in these cells, SMC1/3 signal becomes visible as thread-like staining that coincides with the SC marker C(3)G (Fig. 1D). During the maturation of cysts and their progression through the germarium, thread-like SMC1/3 staining mimics that of the SC (Fig. 1D). As cysts move through the germarium, continuous linear SMC1/3 staining is visible in the two nuclei that contain full-length SC (pro-oocytes) but the SMC1/3 signal appears fragmented in the other C(3)G containing nuclei that will adopt a nurse cell fate (pro-nurse cells). Oocyte determination is complete by region 3 and, at this stage, the continuous thread-like SMC1/3 staining is restricted to the oocyte (Fig. 1D).

The oocyte nucleus will remain in pachytene for several hours as it progresses through the vitellarium (see Fig. 1A). Electron microscopy has shown that that full-length tripartite SC is present as late as stage 6 of vitellarial development (Carpenter, 1975) and these data have been supported by persistence of continuous threads of C(3)G immunostaining until the same stage (Page and Hawley, 2001). In vitellarial stages 2 to 6, we observe thread-like SMC1/3 staining in whole-mount preparations that is coincident with C(3)G signal. However, similar to C(3)G staining, the thread-like signal becomes weaker in these later stages and is accompanied by increased diffuse nuclear staining (see supplementary material Fig. S3).

Cohesin SMCs and ORD are present along chromosome cores during pachytene

Thread-like signals for cohesin SMCs as well as the cohesion protein ORD are restricted to germ-line cells that form SC (Fig. 1D) (Webber et al., 2004). One possibility is that, together, these proteins contribute to the proteinaceous 'chromosome core' that has been proposed to serve as a scaffold for SC formation (Revenkova and Jessberger, 2006; Stack and Anderson, 2001). If the cohesin complex and ORD are indeed part of the chromosome core, they should persist in the absence of DNA loops in SC-containing nuclei (Pearlman et al., 1992; Smith and Roeder, 1997). To test this hypothesis, we prepared chromosome spreads of germarial cells to visualize proteins bound to meiotic chromosomes. In

this procedure, soluble components are washed away (see supplementary material Fig. S4), leaving only chromosomes and their associated proteins attached to the slide. DNase I treatment of chromosome spread slides resulted in loss of histone and DAPI staining, confirming that DNA loops had been digested (Fig. 2). However, the thread-like SMC1/3 and ORD staining persisted in the absence of DNA loops, consistent with the model that ORD and the cohesin complex are components of the cores of meiotic chromosomes in SC-forming nuclei (Fig. 2).

Chromosome spread experiments also revealed that cohesin SMCs are associated with chromosome arms in all 16 nuclei of each germ-line cyst (Fig. 3A). However, in nuclei that do not build a SC, the SMC localization pattern is diffuse rather than thread-like (Fig. 3A, open arrows). In addition, we observed that during SC disassembly in non-oocyte nuclei, cohesin SMCs remain associated with chromosome arms and their staining pattern is indistinguishable from other pro-nurse cells (Fig. 3B). Association of ORD with chromosome arms in a pattern similar to SMC1/3 has been described previously (Webber et al., 2004). Confirmation that soluble nuclear proteins are removed during the spread preparation is shown in supplementary material Fig. S4. When transgenic flies expressing GFP-nls were used to generate spreads, diffuse SMC1 staining was visible in several nuclei but no corresponding GFP signal was detected (see supplementary material Fig. S4). Therefore, we conclude that the diffuse SMC1/3 staining we observe in chromosome spreads represents cohesin SMCs stably associated with the chromatin. These data support the model that cohesin SMCs and ORD associate with the arms of all germ-line chromosomes and in cells that build SC, cohesion proteins coalesce into continuous threads that represent chromosome cores. Interestingly, diffuse SMC staining is often not visible in spread preparations of SC containing nuclei (see Figs 2 and 4), suggesting that most or all of the cohesin complex in these cells is located along the cores, not the loops of meiotic chromosomes.

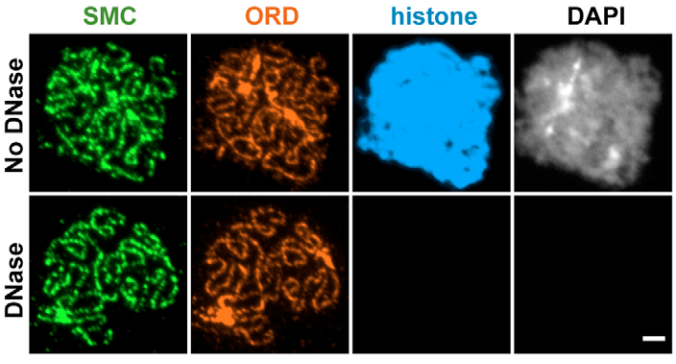


Fig. 2. Cohesin SMCs localize along chromosome cores of meiotic chromosomes. A chromosome spread slide was treated with buffer alone (top panels) or with DNase I (bottom panels) and immunostained for SMC1/3 (green), ORD (orange), histone (blue) and DNA (white). Although histone and DAPI staining is lost when chromosome loops are digested with DNase I, SMC1/3 and ORD signals remain visible along chromosome cores. For each fluorophore, identical exposure times were used to capture cells treated with DNase and not treated with DNase. Bar, 2 μm.

Enrichment of cohesin SMCs at the centromeres of meiotic chromosomes

In addition to the thread-like staining pattern in pachytene cells, cohesin SMCs are enriched at the centromeres of wild-type meiotic chromosomes (Fig. 4, top panels) as confirmed by colocalization of the SMC1/3 foci with CID, the centromere specific histone H3 variant (Blower et al., 2002; Henikoff et al., 2000). The centromeres of *Drosophila* chromosomes are usually clustered together into a single chromocenter (Carpenter, 1975) and each bright focus of SMC1/3 and ORD staining that is visible in the nuclei of whole-mount preparations corresponds to the chromocenter (Fig. 1D). In chromosome spreads, we frequently observe the chromocenter split into two or more regions. Interestingly, the increased resolution afforded by spread preparations indicates that the bright SMC1/3 signal at centromeres often extends beyond the area of CID staining (Fig. 4, see inset). Cohesin SMCs exhibit the same extensive centromeric localization pattern as ORD (Webber et al., 2004), consistent with enrichment of these cohesion proteins within pericentric as well as centromeric heterochromatin. Robust SMC1/3 and ORD signals in the vicinity of the centromere are not restricted to nuclei that build SC. Instead, centromeric enrichment of these proteins is visible in all 16 cells of germarial cysts whether they adopt a nurse cell or oocyte fate (Fig. 3 solid arrows) (Webber et al., 2004).

As egg chambers progress into the vitellarium, the SMC1/3 signal associated with the chromocenter in nurse cells begins to assume a very distinctive pattern that resembles a cluster of finger-like projections (Fig. 5 top, inset). Interestingly, the onset of this staining pattern coincides with the beginning of the endo-reduplication cell cycle in nurse cells, during which DNA replication occurs repeatedly in the absence of cell division. We observe these SMC1/3 finger-like projections during early vitellarial stages when the polyploid nurse cell chromosomes exhibit polyteny, the precise alignment of multiple copies of sister chromatids (King et al., 1981). Unlike polytene chromosomes in the *Drosophila* salivary gland, nurse cell polytene chromosomes are short-lived. Around vitellarial stage 4, nurse cell chromosomes undergo a dramatic morphological change and no longer exhibit polyteny (Dej and Spradling, 1999). Although the SMC1/3 signal remains enriched at the pericentric heterochromatin as nurse cell chromosomes transition out of polyteny, the pattern becomes more diffuse and less structured in these later stages (data not shown).

ORD is required for centromeric localization of cohesin SMCs during meiosis

ORD protein is necessary for both arm and centromeric cohesion during *Drosophila* meiosis (Bickel et al., 1996; Bickel et al., 1997; Mason, 1976). In mutant flies lacking ORD activity, chromosomes segregate randomly through both meiotic divisions, indicating that cohesion is completely absent (Bickel et al., 1996; Bickel et al., 1997; Mason, 1976). The localization pattern of ORD protein during early oogenesis (Webber et al., 2004) closely mimics that of the cohesin SMC proteins. One possibility is that ORD controls the localization and/or function of the cohesin complex during meiosis. To study the localization dynamics of the cohesin complex in the absence of ORD, ovaries from ord^{5}/Df (ord^{null}) females were examined. The ord^{5} mutation results in

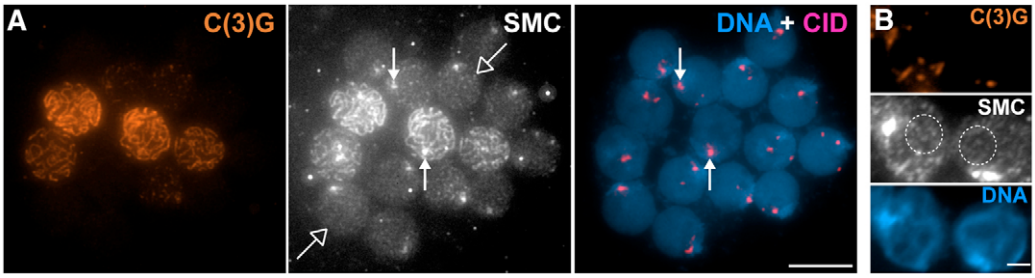


Fig. 3. Cohesin SMCs are associated with centromeres and chromosome arms in all 16 cells of each germ-line cyst. (A) A partial region 2A cyst from a wild-type chromosome spread preparation is stained for C(3)G (orange), SMC1/3 (white), DNA (blue) and CID (pink). SMC1/3 is enriched at the centromeres of all nuclei (solid arrows). In nuclei that contain C(3)G staining, SMC1/3 signal is thread-like. In pro-nurse cell nuclei that do not build SC, diffuse SMC1/3 staining indicates that cohesin subunits are associated with chromatin throughout the nuclei (open arrows). Bar, 5 μm. (B) Wild-type chromosome spread preparation shows that when the SC disassembles in a pro-nurse cell (left nucleus with only short linear stretches of C(3)G staining remaining), the diffuse SMC1/3 signal is similar in pattern and intensity to a nearby nucleus that does not contain appreciable C(3)G signal. Compare SMC1/3 staining within the two circles. Bar, 2 μm.

premature truncation of the ORD open reading frame and genetically behaves like a null-allele (Bickel et al., 1996; Bickel et al., 1997).

When whole-mount *ord*^{null} germaria are stained with SMC1 and SMC3 antibodies, bright centromeric foci are conspicuously absent throughout the germaria even though continuous thread-like staining is visible in region 2A (Fig. 6 and supplementary material Figs S5 and S6). Within the germarium, SMC1/3 foci are undetectable in cells that form SC as well as the remaining cells of each cyst (see supplementary material Figs S5 and S6). *ord*^{null} oocytes also lack SMC1/3 centromeric foci after they exit the germarium (Fig. 5 bottom, open arrow). These data suggest that ORD is essential for normal accumulation of cohesin at oocyte centromeres and are consistent with the chromosome segregation defects observed in mutant flies (Bickel et al., 1996; Bickel et al., 1997; Miyazaki and Orr-Weaver, 1992). Absence of cohesin SMC localization at centromeres in the *ord* mutant was confirmed when chromosome spreads were immunostained for SMC1/3 and the centromere marker CID (Fig. 4). Cohesin SMCs do not colocalize with CID foci in *ord*^{null} germaria; the CID signal corresponds to gaps in the thread-like SMC1/3 signal (Fig. 4, bottom panels). These data suggest that ORD activity is required for loading and/or accumulation of centromeric cohesin during female meiosis.

Interestingly, although centromeric SMC1/3 staining is never visible in oocytes of *ord*^{null} flies, a distinct centromeric staining pattern becomes detectable in nurse cells as cysts progress into the vitellarium. Even in the absence of ORD, we observe finger-like projections of SMC1/3 staining in the vicinity of nurse cell centromeres in *ord*^{null} mutant egg chambers by vitellarial stage 3, presumably when polytene chromosomes are present (Fig. 5 bottom, solid arrow). Like wild type, this SMC1/3 staining becomes diffuse at later stages when polyteny is absent. These data argue that loading of cohesin subunits onto centromeres is controlled differently in oocytes and nurse cells, and once germ-line cells adopt a nurse cell fate, accumulation of cohesin at centromeres is no longer dependent on ORD function.

ORD is necessary for maintenance of chromosome cores during early meiosis

Despite the centromeric defects that we observe in *ord*^{null} germaria, thread-like SMC1 and SMC3 staining along chromosome cores appear relatively normal during early pachytene even in the absence of ORD activity (Fig. 6). In early

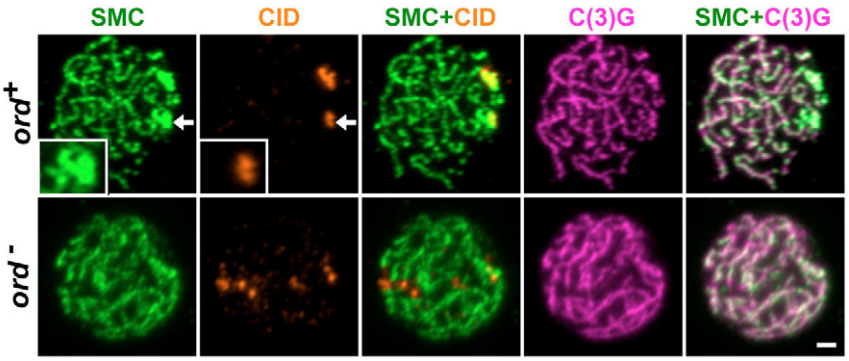


Fig. 4. ORD is required for centromeric localization of cohesin SMCs. (Top panels) Wild-type chromosome spread preparation immunostained for SMC1/3 (green), CID (orange) and C(3)G (magenta). Note that the two bright SMC1/3 patches overlap with the CID signal at the centromeres and thread-like SMC and C(3)G signals are visible along chromosome arms. Insets contain an enlarged view of the pericentric region (arrow) and show that enrichment of SMC1/3 staining extends beyond the CID signal. (Bottom panels) Single nucleus from a *ord*^{null} chromosome spread preparation immunostained for SMC1/3 (green), CID (orange) and C(3)G (magenta). Distinct gaps in the thread-like SMC signal correspond to CID foci, indicating that – in the absence of ORD – cohesin SMCs fail to accumulate at meiotic centromeres. Bar, 2 μm.

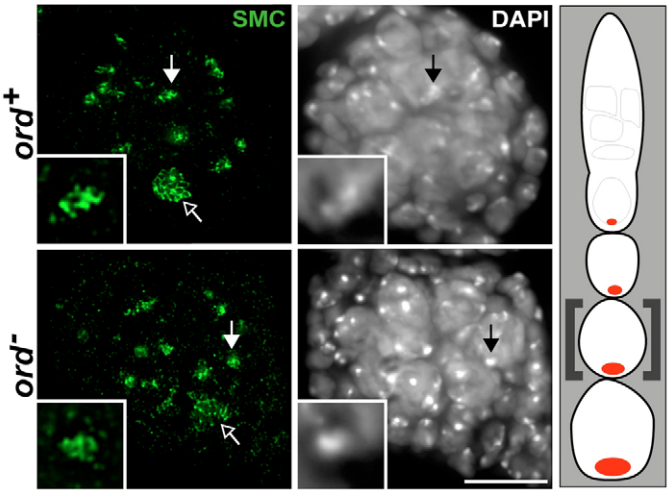


Fig. 5. Cohesin SMCs exhibit a distinct localization pattern at the centromeres of nurse cell chromosomes that is not dependent on ORD. Vitellarial stage-3 egg chambers (see schematic on right) from a whole-mount *ord*$^{+}$ ovariole (top panels) and an *ord*^{null} ovariole (bottom panels) were immunostained for SMC1/3 (green) and DNA (white). Note that SMC staining in both wild-type and mutant nurse cell nuclei coincides with bright DAPI-stained regions (solid arrows). Insets correspond to a magnified view of each region indicated by a solid arrow. Note the SMC1/3 finger-like projections. Open arrows indicate SMC1/3 staining in the oocyte nucleus. All images are projections of deconvolved *Z*-series. The insets include only a subset of sections shown for the entire egg chamber. Bar, 10 μm. The schematic on the right indicates the stage of the cysts shown.

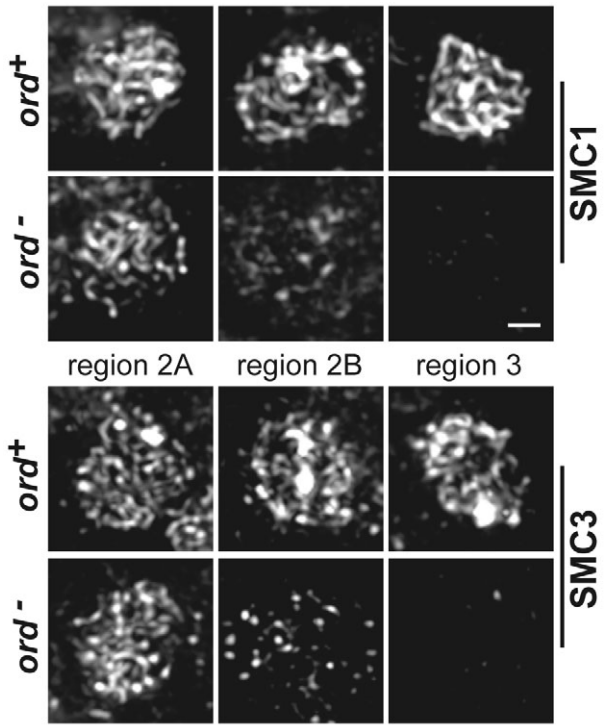


Fig. 6. Chromosome cores disassemble in the absence of ORD activity. SMC1 (top two rows of panels) and SMC3 (bottom two rows panels) immunostaining are shown for single *ord*$^{+}$ and *ord*^{null} nuclei within indicated regions of the germarium. In early region 2A, continuous SMC1 and SMC3 threads are visible in both mutant and wild type. As cysts mature, thread-like SMC staining becomes severely disrupted in the mutant. Only dim fragments or puncta are observed in region 2B *ord*^{null} oocytes, and by region 3 mutant nuclei often contain no visible signal. Note that *ord*^{null} nuclei lack bright SMC1 and SMC3 foci. Images are projections of deconvolved *Z*-series of whole-mount preparations. For each antibody, wild-type and mutant images for each stage were captured and processed identically. Bar, 1 μm.

region 2A (the anterior portion of region 2A), long continuous threads of SMC1 and SMC3 are visible in *ord*^{null} germaria, although SMC staining along cores is weaker than in wild type (Fig. 6, Tables 1, 2). However, both the intensity and integrity of cohesin thread-like staining deteriorates progressively as cysts mature and travel through the germarium (Fig. 6). A gradual loss of thread-like C(3)G staining as cysts mature has also been observed in *ord*^{null} germaria (Webber et al., 2004).

To characterize the progressive deterioration of SMC1 and SMC3 thread-like staining in *ord*^{null} germaria, we scored the integrity and intensity of the threads in different regions of wild-type and mutant germaria (Tables 1 and 2). Careful analysis of the defects in several mutant germaria indicated that, by late region 2A (the posterior portion of region 2A), the intensity of the SMC1 and SMC3 thread-like staining was significantly reduced and fragmented threads were visible in a number of cells (Tables 1 and 2). For example, in late region 2A, a pronounced reduction in SMC1 signal intensity was observed in 43% of *ord*^{null} cysts but only 3% of wild-type cysts (Table 1). Similarly, fragmented SMC1 threads were observed in 15% of late region 2A mutant cysts, but no fragmentation was visible at this stage in wild type (Table 1). In older mutant cysts, loss of the thread-like SMC1 and SMC3 staining became more prominent (Fig. 6). By region 3, no mutant oocyte nucleus exhibited robust continuous thread-like SMC1 or SMC3 staining (n=64 and 48, respectively). At this stage, 45% of *ord*^{null} oocyte nuclei contained no visible SMC1 staining and 53% contained severely fragmented threads (Table 1, Fig. 6). SMC3 signal was undetectable in approximately 52% of region 3 *ord*^{null} oocyte nuclei and about 30% had short dim fragments (Table 2, Fig. 6). Interestingly, the anti-SMC1 and anti-SMC3 antibodies appear to have different affinities for their respective antigens in wild-type nuclei; SMC1 signal along chromosome cores was consistently more robust than that for SMC3 (Tables 1, 2). This difference may reflect variation in epitope accessibility for the two proteins and is most likely the cause for quantitative differences in the defects observed for SMC1 and SMC3 in mutant germaria (Tables 1, 2). However, deterioration of the thread-like signal followed the same trend for both proteins and reinforces the conclusion that ORD activity is required to maintain chromosome cores during early pachytene. Notably, these defects first become manifest after the onset of homologous recombination, namely the induction of DSBs in region 2A (Jang et al., 2003; Mehrotra and McKim, 2006).

ORD is not required for stable association of cohesin SMCs with chromosome arms during pachytene

The loss of thread-like staining in whole-mount preparations of *ord*^{null} germaria initially suggested to us that cohesin

Table 1. SMC1 localization in WT and *ord* germaria

Wild-type cysts				
Localization pattern (%)*	Early region 2A (*n*=35)	Late region 2A (*n*=34)	Region 2B (*n*=19)	Region 3 (*n*=35)
Thread-like†	85.7	97.1	94.4	91.4
Dim threads	14.3	2.9	5.6	8.6
Fragments‡	0.0	0.0	0.0	0.0
Blank§	0.0	0.0	0.0	0.0
ord null¶ cysts				
Localization pattern (%)*	Early region 2A (*n*=64)	Late region 2A (*n*=63)	Region 2B (*n*=54)	Region 3 (*n*=64)
Thread-like†	70.3	42.6	0.0	0.0
Dim threads	29.7	42.6	22.2	1.6
Fragments‡	0.0	14.8	72.2	53.2
Blank§	0.0	0.0	5.6	45.2

*Cysts exhibiting each localization pattern (in %). †Thread-like denotes long continuous staining. ‡Values include fragmented and/or punctate signal. §No visible staining. ¶*ord*^{5}*/Df* flies completely lack ORD activity.

dissociates from chromosome arms during pachytene in the absence of ORD activity, consistent with the essential role of ORD in arm cohesion. However, we reasoned that it was also possible that cohesin SMCs might remain associated with chromatid arms in the absence of ORD, but loss of thread-like staining might occur because the longitudinal compaction of meiotic chromosome cores depends on ORD function. If cohesin SMCs remain associated with chromosome arms during pachytene in *ord*^{null} females but chromosome cores are unstable, the thread-like SMC1/3 signal would disappear. However, it is difficult to detect diffuse localization of cohesin SMCs along chromosome arms in whole-mount preparations. Therefore, we prepared chromosome spreads from *ord*^{null} germaria and immunostained for SMC1/3 and C(3)G proteins (Fig. 7). Because in spread preparations, the temporal arrangement of individual cysts within each germaria is not maintained, we searched for semi-intact cysts that contained a maximum of one or two nuclei with C(3)G staining, reasoning that these most probably represent region 2B cysts. At this stage in *ord*^{null} germaria, C(3)G thread-like signal has begun to fragment (Webber et al., 2004). As shown in Fig. 7, we found that diffuse SMC1/3 staining is readily evident in nuclei that also contain fragmented C(3)G and SMC1/3 threads. Moreover, the intensity of diffuse SMC1/3 signal in these nuclei is very similar to that of adjacent pro-nurse cell nuclei (Fig. 7, see circles). Because soluble nuclear protein is removed during the spread preparation (supplementary material Fig. S4), the diffuse SMC1/3 signal that we observe represents cohesin subunits that are associated with the chromatin but not organized into chromosome cores. These data argue that, in the absence of ORD activity, chromosome cores disassemble but cohesin SMCs remain associated with chromosome arms.

Temporal relationship between SMC1/3 and C(3)G defects in ord^{null} oocytes

The progressive deterioration of chromosome cores in *ord*^{null} germaria is reminiscent of the fragmentation and loss of thread-like staining observed by Webber et al. (Webber et al., 2004) for the SC central element component, C(3)G. However, a careful comparison of the quantitative analyses of cohesin SMC and C(3)G localization defects (this paper) (Webber et al., 2004) indicate that the onset of SMC1/3 localization defects appear to precede those for C(3)G (Fig. 8A). This is not completely unexpected given that chromosome cores have been proposed to serve as the scaffold upon which SC axial/lateral and central element components can assemble. If disruption of chromosome cores in *ord*^{null} oocytes causes a subsequent loss of the central element between homologues, defects in the C(3)G staining pattern should closely follow

Table 2. SMC3 localization in WT and *ord* germaria

Wild-type cysts				
Localization pattern (%)*	Early region 2A (*n*=37)	Late region 2A (*n*=37)	Region 2B (*n*=27)	Region 3 (*n*=36)
Thread-like†	40.0	40.5	3.6	42.9
Dim threads	60.0	59.5	82.1	51.4
Fragments‡	0.0	0.0	14.3	5.7
Blank§	0.0	0.0	0.0	0.0
ord null¢ cysts				
Localization pattern (%)*	Early region 2A (*n*=56)	Late region 2A (*n*=58)	Region 2B (*n*=48)	Region 3 (*n*=48)
Thread-like†	8.6	5.1	0.0	0.0
Dim threads	89.7	83.0	17.4	17.4
Fragments‡	1.7	6.8	30.4	30.4
Blank§	0.0	5.1	52.2	52.2

*Cysts exhibiting each localization pattern (in %). †Thread-like denotes long continuous staining. ‡Values include fragmented and/or punctate signal. §No visible staining. ¶*ord*^{5}*/Df* flies completely lack ORD activity.

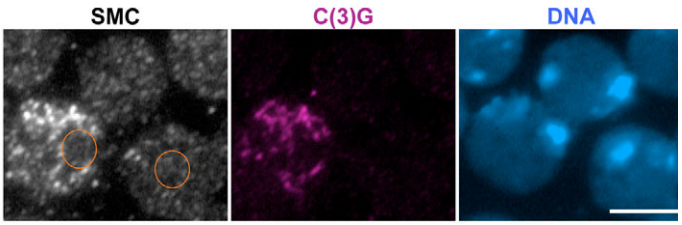


Fig. 7. Cohesin SMCs remain associated with chromosome arms when cores break down prematurely in the absence of ORD activity. Shown is a chromosome spread from the ovary of an *ord*^{null} female stained for SMC1/3 (white), C(3)G (magenta) and DNA (blue). In a nucleus that contains fragmented SMC1/3 and C(3)G staining (left), diffuse chromatin-associated SMC1/3 signal is still visible, indicating that SMCs remain associated with chromosome arms when cores break down in the absence of ORD activity. Note that diffuse cohesin SMC signal in this nucleus is similar in intensity to the SMC1/3 staining in the adjacent pro-nurse cell that did not build SC (compare signal intensity within the two circles). Bar, 5 μm.

disintegration of SMC1/3 threads. To test this hypothesis, SMC1/3 and C(3)G defects were examined simultaneously in individual nuclei at different stages in whole-mount preparations of intact *ord*^{null} germaria.

As shown in Fig. 8B, continuous thread-like staining is evident for both C(3)G and SMC1/3 in early region 2A nuclei of *ord*^{null} germaria with extensive overlap between the two signals. However, by late region 2A, the SMC1/3 signal appears more fragmented than the C(3)G signal, with fewer SMC1/3 threads and more punctate staining. This difference is most obvious in region 3, where intact threads of C(3)G staining are still evident but the SMC1/3 pattern consists primarily of puncta. These data support our hypothesis that premature breakdown of chromosome cores induces the defects in C(3)G staining that we observe in *ord* germaria. However, the residual C(3)G threads that remain when SMC1/3 thread-like staining disappears raises the intriguing possibility that aligned C(3)G proteins might form polymers that remain transiently stable, even if they are no longer associated with chromosome cores.

We also asked whether C(3)G is required to maintain chromosome core integrity and found that thread-like SMC1/3 staining is still visible in *c(3)G*^{68}*/Df* mutant germaria in which the SC fails to form (see supplementary material Fig. S7). These data indicate that intact SC is not necessary for chromosome core formation or maintenance. Interestingly, SMC1/3 threads appear more numerous in *c(3)G* mutant nuclei than in wild type, consistent with the inability of homologues to synapse in the absence of C(3)G. In the absence of synapsis, the homologous cores would not be intimately associated and cohesin staining would be visible along individual chromosome cores. A similar staining pattern has been reported for the putative lateral element component C(2)M in *c(3)G* mutants (Manheim and McKim, 2003) providing further support for this model.

C(2)M is required for chromosome core formation during pachytene

To explore the mechanistic interplay between cohesion proteins (ORD, cohesin SMCs), and an α-kleisin involved in

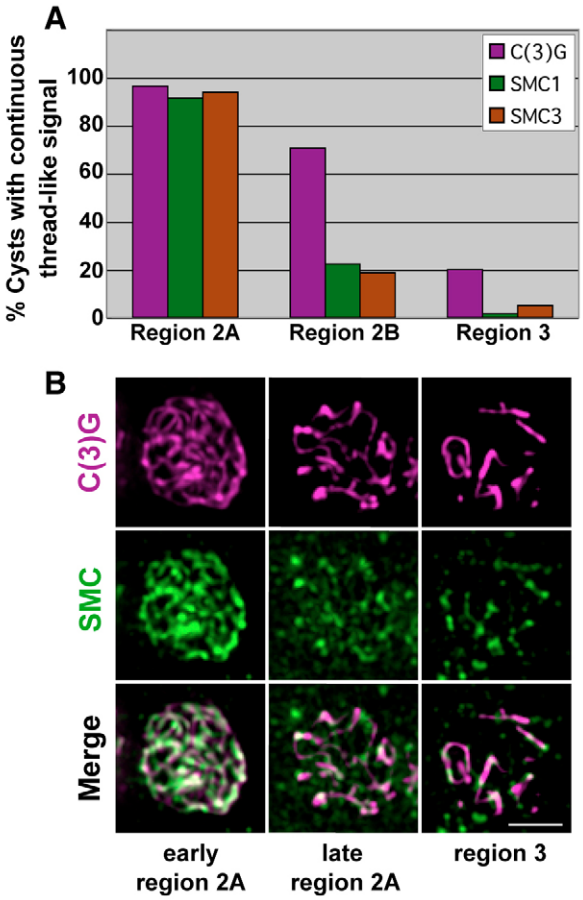


Fig. 8. Fragmentation of chromosome cores is detectable before C(3)G localization defects become apparent in *ord*^{null} germaria. (A) Graph comparing SMC1 and SMC3 localization defects in *ord*^{null} germaria (see Tables 1 and 2) with those observed for C(3)G (Webber et al., 2004). (B) Individual nuclei from whole-mount preparations of *ord*^{null} germaria were simultaneously immunostained for SMC1/3 (green) and C(3)G (magenta). In early region 2A, cohesin SMC and C(3)G signals coincide extensively. Although long stretches of thread-like C(3)G staining are still visible in late region 2A cysts, SMC1/3 staining is severely fragmented. When fragmentation of C(3)G becomes apparent, SMC staining is limited to very short fragments and puncta. Images are projections of deconvolved *Z*-series. SMC images from different stages were captured and processed identically as were the C(3)G images from late region 2A and region 2B. Bar, 2 μm.

SC assembly (C(2)M), we examined the localization of SMC1/3 and GFP-ORD in females homozygous for the *c(2)M*-null allele, *c(2)M*$^{EP[2115]}$ (Manheim and McKim, 2003). To observe ORD localization, we generated a *c(2)M*$^{EP[2115]}$ stock homozygous for a functional GFP-ORD transgene (Balicky et al., 2002) and confirmed that X-chromosome meiotic nondisjunction in *c(2)M*$^{EP[2115]}$*;P{GFP-ORD}* females (24.44%, *n*=753) was similar to that previously reported for *c(2)M*$^{EP[2115]}$ homozygotes (29.3%) (Manheim and McKim, 2003).

At first glance, the staining pattern for SMC1/3 and GFP-ORD in *c(2)M*$^{[EP2115]}$ germaria appeared very similar to that previously observed for C(3)G in this mutant (Manheim and

McKim, 2003). Thread-like SMC1/3 staining is completely absent in the germaria of whole-mount ovaries (see supplementary material Fig. S8). Instead, SMC1/3 staining is restricted to patches and foci.

In *c(2)M*$^{[EP2115]}$ females, the chromosome segregation defects are severe in meiosis I, but negligible during meiosis II (Manheim and McKim, 2003). This suggests that centromeric cohesion in these mutants is intact. To test whether the patches of SMC1/3 and GFP-ORD staining correspond to centromeres, *c(2)M*$^{[EP2115]}$ ovaries were co-immunostained with anti-CID antibodies. In these whole-mount preparations, CID foci largely coincide with ORD and SMC1/3 patches in pro-oocyte and oocyte nuclei in all regions of *c(2)M*-mutant germaria (Fig. 9A). These data argue that neither loading nor maintenance of cohesin SMCs at centromeres depends on the activity of C(2)M protein, consistent with low levels of meiosis II segregation defects in *c(2)M*-mutant females (Manheim and McKim, 2003).

Absence of thread-like SMC1/3 signal in whole-mount preparations of *c(2)M*-mutant germaria raises the possibility that C(2)M activity is required for loading cohesin subunits onto chromosome arms. Alternatively, cohesin subunits may localize normally to the arms, but fail to coalesce into chromosome cores in the absence of C(2)M. In this case, diffuse chromatin-bound cohesin signal would probably go undetected in whole-mount ovary preparations. To address this possibility, we examined chromosome spreads prepared from *c(2)M*$^{EP[2115]}$*;P{GFP-ORD}* germaria (Fig. 9B). In the *c(2)M* mutant, each nucleus contained one to four centromeric foci in which SMC1/3 and GFP-ORD always colocalized. These foci also coincided with C(3)G foci in the subset of cells that contained C(3)G signal. This staining pattern is consistent with that observed in whole-mount preparations of *c(2)M* germaria. However, in the chromosome spreads, diffuse chromosomal ORD and SMC1/3 staining also was observed in all mutant c(2)M pro-oocytes and pro-nurse cells (Fig. 9B). Moreover, the intensity of diffuse SMC1/3 and ORD signal is comparable in nuclei with and without C(3)G patches/foci (pro-oocytes and pro-nurse cells, respectively).

The data for chromosome spread localization indicate that in the absence of C(2)M, ORD and cohesin SMCs are loaded and maintained on both the arms and centromeres of meiotic chromosomes. However, absence of thread-like ORD and SMC1/3 staining argues that assembly of chromosome cores requires C(2)M activity. Because stable chromosome cores and lateral elements are a prerequisite for SC formation, their absence most probably explains the lack of thread-like C(3)G signal in *c(2)M* mutant germaria.

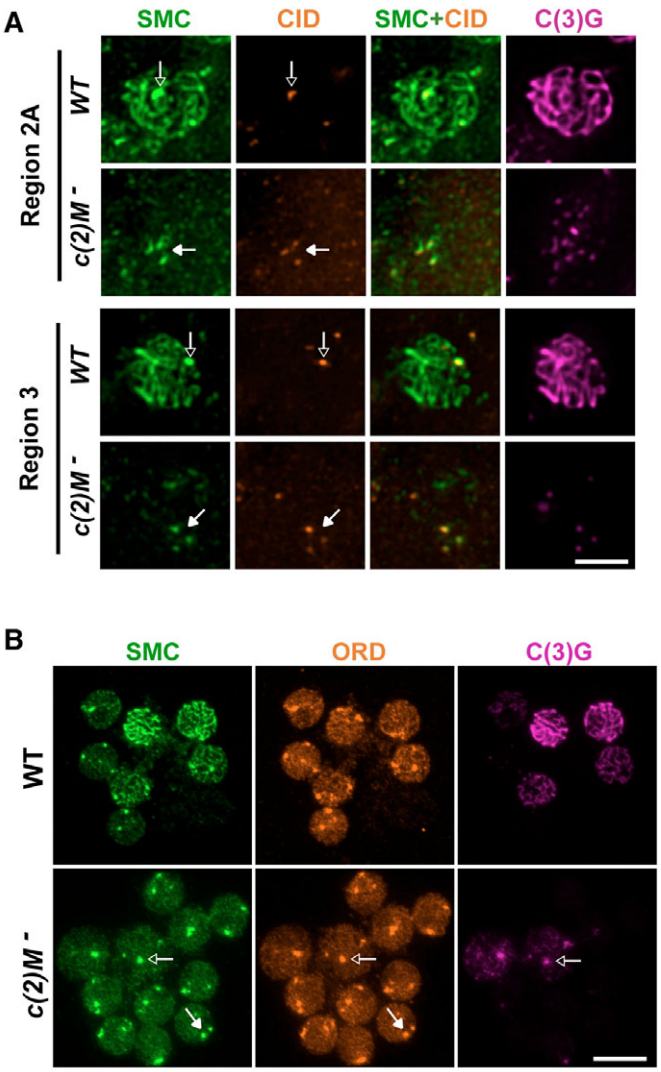


Fig. 9. C(2)M activity is not required for association of cohesin SMCs with chromosome arms or centromeres. (A) Single nuclei from wild-type (WT) and *c(2)M*$^{EP[2115]}$ whole-mount preparations immunostained for SMC1/3 (green), CID (orange), and C(3)G (magenta). In wild-type region 2A and region 3 nuclei, the brightest spots of SMC1/3 staining correspond to the CID signal that marks the centromeres (open arrows). Although chromosome cores are absent in *c(2)M* mutant germaria, patches/foci of SMC1/3 still coincide with CID (solid arrows), indicating that cohesin SMCs localize to centromeres in the absence of C(2)M function. Images are projections of a deconvolved *Z*-series. Bar, 2 μm. (B) Germarial chromosome spread preparations from wild-type (WT) and *c(2)M*$^{EP[2115]}$ mutant (*c(2)M*$^{-/-}$) females expressing GFP-ORD were immunostained for SMC1/3 (green), GFP-ORD (orange), and C(3)G (magenta). In *c(2)M* mutants, all nuclei display diffuse SMC1/3 and ORD signal that is similar to that of WT pro-nurse cell nuclei that do not assemble SC. Note also that all *c(2)M* mutant nuclei exhibit enrichment of SMC1/3 and ORD signal at the centromeres of pro-oocytes and pro-nurse cells (open and solid arrows, respectively). Bar, 10 μm.

Discussion

Here, we describe temporal and spatial changes in cohesin localization during early prophase in wild-type *Drosophila* ovaries as well as in mutants with compromised cohesion and/or homologous recombination. *Drosophila* oogenesis provides a unique opportunity to examine important changes in chromosome morphology that occur during meiotic prophase. Because not all germ-line cells adopt an oocyte fate, the 16-cell cyst allows direct comparison of nuclei that assemble meiotic chromosome cores (and SC) with those that do not. Importantly, this dynamic transformation in chromosome structure depends upon and must occur within the context of functional sister-chromatid cohesion. In addition, these events are crucial for homologous recombination and, therefore, accurate segregation of meiotic chromosomes.

The formation and maintenance of meiotic chromosome cores

Our analysis of chromosome spread preparations from wild-type ovaries indicates that the cohesin subunits SMC1 and SMC3 localize along the arms of chromosomes in all 16 cells of each cyst. However, thread-like cohesin SMC staining is only observed in the nuclei that build a SC. The simplest model to explain the differences we observe in pro-nurse cells and pro-oocytes is that multiple cohesin complexes come together to form long continuous threads of cohesin staining in nuclei that build SC (Fig. 10). We propose that chromosomes in pro-nurse cells maintain an extended interphase-like organization in which cohesin complexes localize along the arms but fail to assemble into this higher order structure. By contrast, the formation of chromosome cores in a subset of nuclei occurs when multiple cohesin complexes along the arms coalesce into threads and, thereby, bring about the shortening of the longitudinal axes of meiotic chromosomes (Fig. 10).

Formation of chromosome cores represents the first step in the organized assembly of the SC. Although DSBs are required for synapsis of homologues in a number of species (Giroux et al., 1989; Grelon et al., 2001; Mahadevaiah et al., 2001), lateral elements are still visible in mutants that fail to make DSBs and therefore lack tripartite SC (Bhuiyan and Schmekel, 2004; Romanienko and Camerini-Otero, 2000). Moreover, chromosome cores have been proposed to serve as the scaffold upon which SC components organize and assemble (Revenkova and Jessberger, 2006; Stack and Anderson, 2001). Genetic and cytological analyses in a number of organisms have confirmed that the cohesin complex plays an integral role in SC assembly (Cai et al., 2003; Klein et al., 1999; Molnar et al., 1995; Pasierbek et al., 2001; Petronczki et al., 2003; Revenkova et al., 2004). Our work argues that cohesin SMCs as well as the cohesion protein ORD are stable components of meiotic chromosome cores, which remain intact when DNase I treatment removes chromatin loops (Pearlman et al., 1992; Smith and Roeder, 1997). Interestingly, in chromosome spread preparations, localization of cohesin subunits and ORD appears to be restricted to chromosome cores; diffuse staining is not detectable in the areas between threads. These data are consistent with the model that, in *Drosophila* meiotic cells, cohesion proteins localize predominantly along the chromatid axes and are not found decorating the loops (Fig. 10). Similar arguments have been made for cohesin localization in *S. cerevisiae* and mouse meiotic cells (Ding et al., 2006; Prieto et al., 2001; Revenkova and Jessberger, 2005).

Here, we provide evidence that the meiosis-specific protein, C(2)M, is required for chromosome core formation in *Drosophila* oocytes. Chromosome spread preparations indicate that in the absence of C(2)M activity, SMC1 and SMC3 diffuse staining is visible throughout the nuclei of all 16 cells within each cyst, indicative of the association of cohesin subunits with chromosome arms. However, no thread-like SMC1/3 or ORD staining is observed in *c(2)M*-mutant germaria. These data indicate that C(2)M protein controls an early step in the formation of chromosome cores. We propose that, by virtue of its ability to interact with cohesin SMC proteins (Heidmann et al., 2004), C(2)M drives the association of cohesin complexes to form meiotic chromosome cores (Fig. 10). Failure in this process would prohibit subsequent assembly of the SC in *c(2)M* mutant germaria as evidenced by lack of thread-like immunostaining for the transverse filament protein C(3)G (Manheim and McKim, 2003). These results also are consistent with EM localization of C(2)M protein along the lateral elements of the wild-type *Drosophila* SC (Anderson et al., 2005).

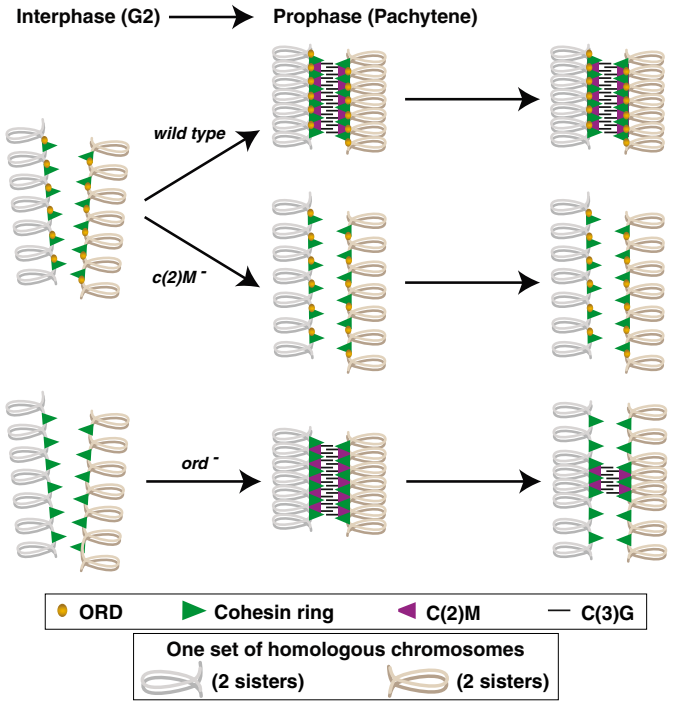


Fig. 10. Model for chromosome core assembly and maintenance. In wild-type meiotic prophase, cohesin proteins localize along the axes of pairs of sister chromatids. Cohesin complexes are brought together by C(2)M, resulting in the formation of visible chromosome cores that provide a scaffold for SC assembly. In the absence of ORD activity, cohesin SMC subunits still associate stably with chromosome arms and chromosome cores can assembly transiently, but are not maintained.

Surprisingly, we found that the cohesion protein ORD is required for the maintenance of chromosome cores during early pachytene in *Drosophila* (Fig. 10). In the absence of ORD activity, thread-like SMC1/3 staining is visible in region 2A of the germarium; however, the intensity and integrity of SMC1/3 threads deteriorate as pachytene progresses within the germarium. Analysis of chromosome spread preparations indicates that, although chromosome cores disassemble, the cohesin subunits SMC1 and SMC3 remain associated with the chromosome arms in ord^{null} germaria.

Our quantitative analyses of temporal progression of SMC1/3 and C(3)G defects in *ord* mutant germaria (this study) (Webber et al., 2004), as well as co-immunostaining experiments that simultaneously monitored cohesin subunits and C(3)G in individual ord^{null} nuclei, argue that the onset of chromosome core dissolution precedes fragmentation of thread-like epifluorescent signal for the SC marker, C(3)G. These data demonstrate that initial assembly of cores is not sufficient for stable SC; instead, maintenance of chromosome cores is an ongoing requirement to preserve SC integrity.

Why do cores disassemble in the absence of ORD activity? By immunofluorescence, continuous thread-like C(2)M and C(3)G staining is transiently present during early pachytene in *ord*^{null} germaria; however, at the same stage, normal tripartite SC is not detectable by EM (Webber et al., 2004). Therefore, although the highly organized SC ultrastructure is absent, some aspects of SC assembly still occur in the absence of ORD function [namely recruitment of C(2)M and C(3)G]. These data suggest that ORD is required to recruit additional proteins along the chromosome cores and/or lateral/axial elements that are required for core integrity. Alternatively, ORD itself might be required to maintain C(2)M-mediated organization of the cores into stable structures.

Programmed cycles of stress and relaxation along meiotic chromosomes have been proposed to govern several critical events during prophase (Kleckner et al., 2004). One possibility is that in the absence of ORD function – although chromosome cores assemble – they are unable to withstand normal changes in compression and/or relaxation, and subsequently buckle. Interestingly, Carpenter (Carpenter, 1975) has reported that, during wild-type pachytene, the SC shortens significantly as cysts move through the *Drosophila* germarium. If chromosome cores that assemble in the absence of ORD are inherently unstable, programmed shortening of the SC could cause additional stress that results in fragmentation of the cores. Curiously, we have observed that in later stages (stages 3-6 of the vitellarium), continuous thread-like SMC1/3 staining often reappears in *ord*^{null} oocytes (see Fig. 5). These stages loosely correspond to the time after which the SC reaches its shortest length and starts to expand in wild type. Chromosome cores might be able to reassemble at these later stages in *ord*^{null} ovarioles if decompaction of the chromosome axes reduces stress.

Breakdown of chromosome cores in *ord*^{null} germaria is also temporally linked to the onset of DSBs. Both the timing (early 2A) and the number of DSBs are normal in *ord*^{null} germaria (Webber et al., 2004). However, we begin to detect fragmentation of SMC1/3 thread-like staining in late region 2A, after the onset of DSBs. Therefore, induction of DSBs might contribute to destabilization of chromosome cores that are compromised due to lack of ORD protein. A similar model has been proposed to explain the phenotypes associated with disruption of the Pds5 orthologue (Spo76) in Sordaria (Storlazzi et al., 2003). Although AEs are continuous during early prophase I in *spo76-1* mutants, they fragment prematurely in a DSB-dependent fashion (Storlazzi et al., 2003).

The disassembly of chromosome cores in *ord*^{null} germaria is most probably responsible for the severe reduction in crossovers between homologues in mutant females. ORD activity is essential for the crucial decision each chromatid must make after the induction of DSBs – namely whether the broken chromatid will choose its sister or its homologue for repair. Strand invasion and crossovers are biased towards the homologue during meiosis, resulting in stable chiasmata that keep homologous chromosomes physically associated until anaphase I (Schwacha and Kleckner, 1997; Zickler and Kleckner, 1999). Previous experiments have shown that ORD activity is required for homologue bias during meiotic recombination in *Drosophila*. In *ord*^{null} females, the frequency of crossovers between homologues is decreased, while that between sister chromatids is significantly increased (Webber et al., 2004). These data combined with our current analyses suggest that chromosome cores are necessary for homologue bias during meiosis, and partner choice takes place in late region 2A or region 2B of the *Drosophila* germarium.

Different requirements for arm and centromeric cohesin loading

In *ord*^{null} germaria, SMC1 and SMC3 fail to accumulate at centromeres, but appear to localize normally along chromosome arms within all 16 cells of each germ-line cyst. These results suggest that distinct pathways mediate cohesin loading on the arms and centromeres during *Drosophila* meiosis. We cannot differentiate whether centromeric loading of SMC1 and SMC3 is completely ablated or whether cohesin SMCs are able to load at centromeres but are quickly removed when ORD activity is absent. Regardless, accumulation of cohesin subunits at the centromeres of meiotic chromosomes appears to depend on ORD function. By contrast, ORD activity is not required for stable association of SMC1 and SMC3 along chromosome arms. Curiously, after germ-line cells adopt a nurse cell fate, ORD is no longer necessary for centromeric accumulation of cohesin; the clustered finger-like projections of SMC1/3 staining at nurse cell centromeres in *ord*^{null} ovarioles (stages 3-4) are indistinguishable from that in wild type. However, even at these later stages when cohesin subunits are visible at nurse cell centromeres, we never detect SMC1 or SMC3 at the centromeres of oocytes in *ord*^{null} ovarioles. Therefore, our data implicate ORD in a meiosis-specific pathway for cohesin loading and/or accumulation at centromeres.

Co-immunostaining experiments with CID and SMC1/3 antibodies indicate that absence of cohesin subunits appears to be restricted to the centromeres in *ord*^{null} germaria; within the resolution limits of our chromosome spread images, the area lacking SMC1/3 signal is approximately the same size as the CID staining. These data suggest that cohesin loading and/or accumulation at pericentromeric heterochromatin occurs in the absence of ORD function. However, we do not observe the striking enrichment of SMC1/3 in pericentromeric heterochromatin prominent in wild type. Therefore, our analysis of defects of cohesin localization in *ord*^{null} germaria suggest that normal loading and/or accumulation of cohesin is regulated differently even within the different domains of heterochromatin in and around centromeres. In addition, the chromocenter appears to be less stable in *ord*^{null} oocytes (see Fig. 4), raising the possibility that changes in heterochromatin structure in the absence of ORD activity diminishes the ability of centromeres to associate.

Our chromosome spread experiments clearly indicate that SMC1 and SMC3 are stably associated with the chromosome arms of both pro-nurse cells and pro-oocytes within *ord*^{null} germaria. However, whether the localization of cohesin subunits represents functional cohesin is not clear. From genetic and cytological studies, we know that meiotic cohesion is completely absent in *ord*^{null} oocytes by the time that meiotic chromosomes make microtubule attachments (Bickel et al., 2002; Bickel et al., 1997). Separated sister chromatids have not been detected during early pachytene in *ord*^{null} germaria by FISH (Webber et al., 2004). However, catenation might hold sisters together at this time, thereby masking defects arising

from the absence of cohesin-mediated cohesion (Toyoda and Yanagida, 2006).

Stepwise loading of cohesin subunits during meiotic prophase has been described for a number of organisms (Chan et al., 2003; Eijpe et al., 2003; Valdeolmillos et al., 2007). In worms and grasshoppers, stable association of cohesin SMCs in the absence of non-SMC subunits has been reported for meiotic chromosomes (Chan et al., 2003; Valdeolmillos et al., 2007). One possibility is that, in the absence of ORD, cohesin SMCs load without their non-SMC partners. Another possibility is that the entire cohesin complex loads in *ord*^{null} ovaries but cohesin-mediated cohesion is not established. At least two reports have indicated that, in *S. cerevisiae*, the binding of cohesin to specific genomic locations is insufficient for cohesin-mediated cohesion (Chang et al., 2005; Lam et al., 2006). In addition, recent work by Ellenberg and colleagues (Gerlich et al., 2006) it has been elegantly demonstrated that different populations of chromatin-bound mitotic cohesin exist within Hela cells. This work suggests that, during replication and the establishment of cohesion, a subset of chromatin-bound cohesin complexes is converted from 'dynamically associated' to 'irreversibly bound'. ORD might be necessary for the establishment of meiotic sister-chromatid cohesion but not for association of cohesin subunits with the chromosomes. Alternatively, cohesion might be established in the absence of ORD activity but not maintained.

The identity of *Drosophila* Rec8?

In most species, a meiosis-specific α-kleisin subunit (Rec8) promotes meiotic cohesion by interacting with the heads of the SMC subunits and closing the cohesin ring. Surprisingly, an obvious Rec8 orthologue has not been identified in the *Drosophila* genome. Although its limited sequence homology to Rec8 in other organisms has led to the proposal that C(2)M functions as the meiosis-specific α-kleisin subunit of the cohesin complex in *Drosophila* (Schleiffer et al., 2003), phenotypic analysis of *c(2)M* mutant flies is inconsistent with this hypothesis (Manheim and McKim, 2003). In contrast to other *Drosophila* mutations that disrupt meiotic cohesion (Bickel et al., 1997; Kerrebrock et al., 1992; Miyazaki and Orr-Weaver, 1992), defects of meiosis II segregation are negligible in *c(2)M* females and accurate chromosome segregation during male meiosis also does not depend on C(2)M function (Manheim and McKim, 2003). Moreover, female germ-line expression of a mutated C(2)M transgene in which putative separase cleavage sites were disrupted did not result in meiotic segregation defects (Heidmann et al., 2004). Finally, localization of C(2)M protein in whole-mount preparations (Manheim and McKim, 2003) indicates that, like C(3)G protein, C(2)M is restricted to the subset of cells within each germ-line cyst that build SC. Therefore, we propose that the kleisin domain in C(2)M allows it to interact with cohesin SMC subunits and that C(2)M plays an essential role in building meiotic chromosome cores, but does not promote meiotic cohesion.

In several respects, the behavior of ORD protein is consistent with it performing the role of Rec8 during *Drosophila* meiosis. Null mutations in *ord* eliminate meiotic cohesion in both sexes and ORD colocalizes extensively with cohesin SMC subunits during early pachytene in females. During male meiosis, ORD remains associated with spermatoctye centromeres throughout prophase I (E. M. Balicky, Regulation of chromosome segregation by ORD and dRING during Drosophila meiosis, PhD thesis, Dartmouth College, Hanover, NH, 2004) and is not lost until anaphase II when centromeric cohesion is released (Balicky et al., 2002). Moreover, retention of ORD at centromeres until anaphase II depends on the activity of the *Drosophila* Shugoshin ortholog, Mei-S332 (E. M. Balicky, PhD thesis, Dartmouth College, Hanover, NH, 2004). We have been unable to detect ORD (or cohesin SMCs) on meiotic chromosomes during late oogenesis; however, given that ORD is required for cohesion in both sexes and localizes to spermatocyte centromeres until anaphase II, it seems likely that lack of signal in mature oocytes is due to antibody accessibility issues and/or detection limitations, not the absence of the protein.

Although several pieces of data are consistent with the model that ORD protein provides Rec8 activity in *Drosophila*, the size of ORD protein (479 amino acids) may be too small to bridge the heads of the *Drosophila* SMC1/3 dimer. In addition, ORD does not share obvious sequence homology with Rec8, Scc3/SA or regulators of cohesin (Pds5, Scc4, Scc2). One possibility is that during *Drosophila* meiosis, two proteins collaborate to provide Rec8 function. Such is the case for *Drosophila* separase, which is composed of two subunits encoded by separate genes (Herzig et al., 2002; Jager et al., 2001; Leismann et al., 2000). ORD may cooperate with Rad21 or another unidentified protein to provide Rec8 function during meiosis. Why flies would use an altered mechanism to accomplish such a highly conserved activity is an enigma. However, further analysis of the regulation of meiotic cohesion in *Drosophila* should provide important evolutionary insights into fundamental aspects of recombination and chromosome segregation during meiosis.

Materials and Methods

Fly strains

Flies were reared at 25°C on standard cornmeal molasses medium. *y w; ord*^{10} *bw;* females containing a functional *P{gfp::ord}* transgene (Balicky et al., 2002) were used for immunolocalization of GFP-ORD. For all other *ord*$^{+}$ experiments, *y; cn bw sp* females were used. *ord*^{null} (*ord*^{5}*/Df*) females were obtained by crossing *y/y*$^{+}$ *Y; ord*^{5} *bw/SM1; spa*^{pol} males (Miyazaki and Orr-Weaver, 1992) to *y; Df(2R)WI370/CyO bw* virgins (Bickel et al., 1996). For experiments with *c(2)M* mutants, homozygous *c(2)M* females from the stock *y w/y*$^{+}$ *Y; c(2)M*^{EP2115}*/SM1; P{gfp::ord}* were used. The *c(2)M*^{EP2115} mutant stock expressing GFP-tagged ORD was generated by crossing *y w;; P{gfp::ord}* females (Balicky et al., 2002) to *y w/y*$^{+}$ *Y; c(2)M*^{EP2115}*/CyO* males (Manheim and McKim, 2003). For experiments with *c(3)G* mutants, *y w/y; c(3)G*^{68} *e/cv-v Df(3R)c(3)G-2; spa*^{pol} females were obtained by crossing *y w;; c(3)G*^{68} *e/TM3 Sb; spa*^{pol} females (Page and Hawley, 2001) to *y/Y;; cv-v Df(3R)c(3)G-2/TM2 P{y*$^{+}$*} Ser e; spa*^{pol} males (Nelson and Szauter, 1992). *smc1*^{exc46} (Dorsett et al., 2005) germ-line clones were generated by crossing *y w;; FRT 82B sr smc1*^{exc46}*/TM3 Sb* males to FLP *y w; P{gfp::ord}; FRT82B LacZ/TM3 Sb* virgins and heat shocking the crosses at 38°C for 1 hour on days 3 and 4. *Sb*$^{+}$ females were selected for immunolocalization experiments. To monitor the behavior of a soluble nuclear protein in the chromosome spread preparation, homozygous females from *w; P{w*$^{+mC}$*=Ubi-GFP(S65T)nls}3L P{ry*$^{+t7.2}$*=neoFRT}80B/TM3 Sb* were used (Bloomington Stock #5630).

Fixation and immunolocalization of whole-mount ovaries

Most immunolocalization experiments were performed as previously described (Page and Hawley, 2001; Webber et al., 2004) except that ovaries were fixed in unbuffered formaldehyde/heptane (200 μl of unbuffered 2% EM-grade formaldehyde containing 0.5% Nonidet P-40 and mixed with 600 μl heptane). Ovaries were incubated in primary antibodies for 2 hours and the antibodies (primary and secondary) were diluted in PBS containing 0.5% BSA and 0.01% Tween-20.

To analyze *smc1*$^{-/-}$ germ-line clones (see supplementary material Fig. S1b), fixation of ovaries and staining with LacZ, SMC1 and C(3)G antibodies was performed using a protocol adapted from Song et al. (Song et al., 2002). Ovaries

were fixed in 4% formaldehyde in 1× PBS for 15 minutes, followed by four washes in 1× PBS containing 0.1% Triton X-100 (Pierce). After blocking overnight in 1×PBS containing 1% BSA and 0.5% normal donkey serum, the ovaries were rinsed 3 times in 1×PBS containing 0.5% BSA and 0.01% Tween-20 (antibody buffer) and incubated overnight in SMC1 and C(3)G antibodies diluted in antibody buffer. After three rinses and three washes in 1×PBS containing 0.2% Tween-20, the ovaries were incubated in appropriate secondary antibodies. Following three rinses and three washes in 1× PBS containing 0.1% Triton X-100, ovaries were incubated overnight in β-galactosidase antibodies diluted in 1×PBS. After three rinses and three washes in 1×PBS containing 0.1% Triton X-100, the ovaries were incubated in appropriate secondary antibodies, followed by three rinses and one wash in 1×PBS containing 0.1% Triton X-100. Ovaries were then stained with DAPI and mounted in Prolong. To observe nuclear GFP fluorescence in whole-mount ovaries (see supplementary material Fig. S4), ovaries were fixed in 4% formaldehyde in 1×PBS for 20 mins. After three rinses in 1×PBS, ovaries were stained with DAPI and mounted in Prolong.

Chromosome spreads

The procedure for preparing *Drosophila* ovary chromosome spreads (Webber et al., 2004) was adapted from a protocol developed for mammalian meiotic cells (Peters et al., 1997). Fourteen young females of the appropriate genotype were fattened overnight with yeast and males, and their ovaries were isolated in PBS. Ovaries were rinsed once in freshly prepared hypotonic buffer (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) and incubated for 20-30 minutes in hypotonic buffer. Tungsten needles were used to isolate the transparent tips of the ovaries in hypotonic buffer. Ovary tips were then transferred to 40 μl of 100 mM sucrose, minced thoroughly using the tungsten needles, and minced tissue was pipetted up and down several times through a BSA-coated P-2 pipette tip with the pipettor set at 10 μl. Of the minced tissue, 10 μl were placed in the center of a slide which had been dipped in fixative (25 ml freshly prepared 1% paraformaldehyde adjusted to pH 9.2 to which 350 μl of 10% Triton X-100 had been added). Tissue was spread onto the slide by tilting and rotating the slide to distribute the liquid. Once all the minced tissue was spread onto slides, slides were placed in a closed humid chamber overnight and allowed to dry slowly. The humid chamber was opened slightly the next day and slides were allowed to dry completely. Dry slides were dipped in a 0.4% solution of Kodak Photoflo 200 for 2 minutes and allowed to air dry at room temperature. Once dry, they were stored at –20°C and used for immunofluorescence within 1-2 days.

Prior to immunolocalization, the spreads were rehydrated in PBS for 15 minutes and blocked for 1 hour in 5% normal donkey serum, 2% BSA, 0.1% Triton X-100, PBS. Slides were rinsed three times with antibody buffer (0.1% BSA, 0.1% Triton X-100, PBS) and 100 μl of antibodies diluted in the antibody buffer were added to the slides and a piece of parafilm was used to cover the liquid on the slide. The primary antibody incubation was performed for 2-4 hours in a humid chamber. Slides were rinsed three times and washed three times for 10 minutes each with wash buffer (0.1% Triton X-100, PBS). Secondary antibody incubations were performed in antibody buffer for 1 hour and after rinsing and washing slides with wash buffer the tissue was stained with DAPI at 1 μg/ml in PBS. After an additional rinse in PBS, the slides were mounted in 40-50 μl of Prolong (Molecular Probes) under 24×50 mm coverslips.

For DNase treatment of chromosome spreads, tissue was rehydrated in PBS for 15 minutes and blocked in 5% donkey serum, 2% BSA, 0.1% Triton X-100, 0.01% Na Azide in PBS. Slides were rinsed three times in DNase buffer (1% BSA, 10 mM $MnCl_2$, 1 mM $CaCl_2$, 50 mM Tris pH 7.5) and incubated for 1 hour at 37°C in 100 μl DNase buffer only or DNase buffer containing 100U/ml of DNase I (Catalog # LS006342, Worthington Biochemical Corp.) under parafilm. Antibody incubations and washes were performed after DNase treatment as described above.

Antibodies

Guinea pig polyclonal antibodies were generated against SMC1 and SMC3 peptides at Alpha Diagnostic International Inc., San Antonio, TX. The SMC1 peptide MTEEDDDVAQRVATAPVRKP corresponds to the N-terminus of the predicted protein (CG6057). The SMC3 peptide CVTREEAKVFVEDDSTHA corresponds to the predicted C-terminus (Cap, CG9802). Serum was affinity-purified against peptides conjugated to Sulfolink resin (Pierce). Eluted antibodies were exchanged into PBS and concentrated using Centricon-30 filtration. An equal volume of glycerol was added before aliquots were stored at –80°C.

Antibody incubations

SMC1 antibodies were diluted 1:2000 for whole-mount experiments and 1:500 for chromosome spreads. SMC3 antibodies were diluted 1:1000 for whole-mount experiments and 1:500 for chromosome spreads. SMC1 and SMC3 primary antibodies were detected using Cy3-conjugated anti-guinea-pig secondary antibodies. In a number of experiments, SMC1 and SMC3 antibodies were mixed (SMC1/3) and used together. For colocalization of SMCs with other proteins, SMC1/3 staining was completed before incubation with other primary antibodies. C(3)G monoclonal antibodies (supernatant from line 1A8-1G2) (Anderson et al., 2005) were used at 1:500 followed by either Alexa Fluor 488-conjugated anti-mouse secondary or Cy5-conjugated anti-mouse secondary. For detection of GFP-ORD, rabbit anti-GFP antibodies (Molecular Probes) were diluted 1:2000 for whole-mount experiments and 1:1000 for chromosome spreads followed by Alexa Fluor 488-conjugated anti-rabbit secondary. Affinity-purified chicken anti-CID antibodies (Blower and Karpen, 2001) were used at 1:100 followed by Cy5 anti-chicken secondary. Anti-CID rabbit antibodies (Abcam) were used at 1:1000 followed by either Alexa Fluor 488-conjugated anti-rabbit secondary or Cy5-conjugated anti-rabbit secondary. 1B1 mouse monoclonal antibodies (Zaccai and Lipshitz, 1996) were used at 1:20 to detect the fusome with Cy5-conjugated anti-mouse secondary. Anti β-galactosidase chicken antibodies (Abcam) were used at 1:2000. Secondary antibodies conjugated to Cy3 and Cy5 were obtained from Jackson Immunoresearch Laboratories, and the Alexa Fluor 488-conjugated secondary antibodies were obtained from Molecular Probes. All secondary antibodies were used at a final dilution of 1:400.

Microscopy and image analysis

Epifluorescence microscopy was performed using a Zeiss Axioplan2 or Zeiss Axioimager M1 microscope equipped with a Hamamatsu ORCA-ER camera. 100× Plan-Apochromat (NA 1.4) and 63× Plan-Apochromat (NA 1.4) objectives were used for imaging whole mounts and spreads, respectively. Images were captured using Openlab software (Improvision, version 3.1.5 and higher). Registration of images for colocalization experiments was performed using Tetraspeck fluorescent beads (Molecular Probes) and the Openlab software registration module. Openlab or Volocity (Improvision) was used to crop and pseudo-color images. Deconvolution of image stacks (0.1 μm step size) was performed using Volocity (version 2.5 and higher). To preserve relative intensities, mutant and wild-type images for each stage were captured using the same exposure time, and deconvolved simultaneously as 'different timepoints' within a single 'image sequence'.

We thank R. Scott Hawley and Scott L. Page for C(3)G monoclonal antibody, *c(3)G*^{68} and *smc1*^{exc46} flies, Kim S. McKim for *c(2)M*^{EP2115} flies, Gary Karpen for affinity-purified CID antibody and the Bloomington Stock Center for supplying the GFP-nls stock. The fusome (1B1) antibody developed by Howard D. Lipshitz (Zaccai and Lipshitz, 1996) was obtained from the U. of Iowa, Developmental Studies Hybridoma Bank developed under the auspices of NICHD. We thank Brian Schneider and Warren Davis (Improvision) for technical support regarding deconvolution, Ann Lavanway for microscopy support, and Christine Maute for help with Fig. 10. Dale Dorsett, Amy Gladfelter and the Bickel lab members (especially Viji Subramanian) provided invaluable comments on the manuscript. We thank Pei Zhou for advice about making germ-line clones. This work was funded by March of Dimes (5-FY98-738) and National Institutes of Health (GM-59354) awards to S.E.B.

References

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F. et al. (2000). The genome sequence of *Drosophila* melanogaster. *Science* **287**, 2185-2195.

Anderson, L. K., Royer, S. M., Page, S. L., McKim, K. S., Lai, A., Lilly, M. A. and Hawley, R. S. (2005). Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of *Drosophila* synaptonemal complex. *Proc. Natl. Acad. Sci. USA* **102**, 4482-4487.

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

Bhuiyan, H. and Schmekel, K. (2004). Meiotic chromosome synapsis in yeast can occur without spo11-induced DNA double-strand breaks. *Genetics* **168**, 775-783.

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

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

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

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

Buonomo, S. B., Rabitsch, K. P., Fuchs, J., Gruber, S., Sullivan, M., Uhlmann, F., Petronczki, M., Toth, A. and Nasmyth, K. (2003). Division of the nucleolus and its release of CDC14 during anaphase of meiosis I depends on separase, SPO12, and SLK19. *Dev. Cell* **4**, 727-739.

Cai, X., Dong, F., Edelmann, R. E. and Makaroff, C. A. (2003). The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. *J. Cell Sci.* **116**, 2999-3007.
Carpenter, A. T. C. (1975). Electron microscopy of meiosis in *Drosophila melanogaster* females. *Chromosoma* **51**, 157-182.
Chan, R. C., Chan, A., Jeon, M., Wu, T. F., Pasqualone, D., Rougvie, A. E. and Meyer, B. J. (2003). Chromosome cohesion is regulated by a clock gene paralogue TIM-1. *Nature* **424**, 1002-1009.
Chang, C. R., Wu, C. S., Hom, Y. and Gartenberg, M. R. (2005). Targeting of cohesin by transcriptionally silent chromatin. *Genes Dev.* **19**, 3031-3042.
Couteau, F., Nabeshima, K., Villeneuve, A. and Zetka, M. (2004). A component of C. elegans meiotic chromosome axes at the interface of homolog alignment, synapsis, nuclear reorganization, and recombination. *Curr. Biol.* **14**, 585-592.
Dej, K. J. and Spradling, A. C. (1999). The endocycle controls nurse cell polytene chromosome structure during *Drosophila* oogenesis. *Development* **126**, 293-303.
Ding, D. Q., Sakurai, N., Katou, Y., Itoh, T., Shirahige, K., Haraguchi, T. and Hiraoka, Y. (2006). Meiotic cohesins modulate chromosome compaction during meiotic prophase in fission yeast. *J. Cell Biol.* **174**, 499-508.
Dorsett, D., Eissenberg, J. C., Misulovin, Z., Martens, A., Redding, B. and McKim, K. (2005). Effects of sister chromatid cohesion proteins on cut gene expression during wing development in *Drosophila*. *Development* **132**, 4743-4753.
Eijpe, M., Offenberg, H., Jessberger, R., Revenkova, E. and Heyting, C. (2003). Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1beta and SMC3. *J. Cell Biol.* **160**, 657-670.
Gerlich, D., Koch, B., Dupeux, F., Peters, J. M. and Ellenberg, J. (2006). Live-cell imaging reveals a stable cohesin-chromatin interaction after but not before DNA replication. *Curr. Biol.* **16**, 1571-1578.
Giroux, C. N., Dresser, M. E. and Tiano, H. F. (1989). Genetic control of chromosome synapsis in yeast meiosis. *Genome* **31**, 88-94.
Grelon, M., Vezon, D., Gendrot, G. and Pelletier, G. (2001). AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J.* **20**, 589-600.
Heidmann, D., Horn, S., Heidmann, S., Schleiffer, A., Nasmyth, K. and Lehner, C. F. (2004). The *Drosophila* meiotic kleisin C(2)M functions before the meiotic divisions. *Chromosoma* **113**, 177-187.
Henikoff, S., Ahmad, K., Platero, J. S. and van Steensel, B. (2000). Heterochromatic deposition of centromeric histone H3-like proteins. *Proc. Natl. Acad. Sci. USA* **97**, 716-721.
Herzig, A., Lehner, C. F. and Heidmann, S. (2002). Proteolytic cleavage of the THR subunit during anaphase limits *Drosophila* separase function. *Genes Dev.* **16**, 2443-2454.
Hodges, C. A., Revenkova, E., Jessberger, R., Hassold, T. J. and Hunt, P. A. (2005). SMC1β-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. *Nat. Genet.* **37**, 1351-1355.
Hong, C. S. and Genetzky, B. (1996). Molecular characterization of neurally expressing genes in the para sodium channel gene cluster of *Drosophila*. *Genetics* **142**, 879-892.
Jager, H., Herzig, A., Lehner, C. F. and Heidmann, S. (2001). *Drosophila* separase is required for sister chromatid separation and binds to PIM and THR. *Genes Dev.* **15**, 2572-2584.
James, R. D., Schmiesing, J. A., Peters, A. H., Yokomori, K. and Disteche, C. M. (2002). Differential association of SMC1alpha and SMC3 proteins with meiotic chromosomes in wild-type and SPO11-deficient male mice. *Chromosome Res.* **10**, 549-560.
Jang, J. K., Sherizen, D. E., Bhagat, R., Manheim, E. A. and McKim, K. S. (2003). Relationship of DNA double-strand breaks to synapsis in *Drosophila*. *J. Cell Sci.* **116**, 3069-3077.
Kerrebrock, A. W., Miyazaki, W. Y., Birnby, D. and Orr-Weaver, T. L. (1992). The *Drosophila mei-S332* gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. *Genetics* **130**, 827-841.
Kerrebrock, A. W., Moore, D. P., Wu, J. S. and Orr-Weaver, T. L. (1995). MEI-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell* **83**, 247-256.
King, R. C. (1970). *Ovarian Development in Drosophila melanogaster*. New York: Academic Press.
King, R. C., Riley, S. F., Cassidy, J. D., White, P. E. and Paik, Y. K. (1981). Giant polytene chromosomes from the ovaries of a *Drosophila* mutant. *Science* **212**, 441-443.
King, R. C., Rubinson, A. C. and Smith, R. F. (1956). Oogenesis in adult *Drosophila* melanogaster. *Growth* **20**, 121-157.
Kleckner, N., Zickler, D., Jones, G. H., Dekker, J., Padmore, R., Henle, J. and Hutchinson, J. (2004). A mechanical basis for chromosome function. *Proc. Natl. Acad. Sci. USA* **101**, 12592-12597.
Klein, F., Mahr, P., Galova, M., Buonomo, S. B., Michaelis, C., Nairz, K. and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* **98**, 91-103.
Lam, W. W., Peterson, E. A., Yeung, M. and Lavoie, B. D. (2006). Condensin is required for chromosome arm cohesion during mitosis. *Genes Dev.* **20**, 2973-2984.
Lee, J. Y. and Orr-Weaver, T. L. (2001). The molecular basis of sister-chromatid cohesion. *Annu. Rev. Cell Dev. Biol.* **17**, 753-777.
Leismann, O., Herzig, A., Heidmann, S. and Lehner, C. F. (2000). Degradation of *Drosophila* PIM regulates sister chromatid separation during mitosis. *Genes Dev.* **14**, 2192-2205.
Mahadevaiah, S. K., Turner, J. M., Baudat, F., Rogakou, E. P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W. M. and Burgoyne, P. S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. *Nat. Genet.* **27**, 271-276.
Manheim, E. A. and McKim, K. S. (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.
McGuinness, B. E., Hirota, T., Kudo, N. R., Peters, J. M. and Nasmyth, K. (2005). Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. *PLoS Biol.* **3**, e86.
McKim, K. S. and Hayashi-Hagihara, A. (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.
Mehrotra, S. and McKim, K. S. (2006). Temporal analysis of meiotic DNA double-strand break formation and repair in *Drosophila* females. *PLoS Genet.* **2**, e200.
Miyazaki, W. Y. and Orr-Weaver, T. L. (1992). Sister-chromatid misbehavior in *Drosophila ord* mutants. *Genetics* **132**, 1047-1061.
Molnar, M., Bahler, J., Sipiczki, M. and Kohli, J. (1995). The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. *Genetics* **141**, 61-73.
Nasmyth, K. (2002). Segregating sister genomes: the molecular biology of chromosome separation. *Science* **297**, 559-565.
Nelson, C. R. and Szauter, P. (1992). Cytogenetic analysis of chromosome region 89A of *Drosophila* melanogaster: isolation of deficiencies and mapping of Po, Aldox-1 and transposon insertions. *Mol. Gen. Genet.* **235**, 11-21.
Page, S. L. and Hawley, R. S. (2001). *c(3)G* encodes a *Drosophila* synaptonemal complex protein. *Genes Dev.* **15**, 3130-3143.
Page, S. L. and Hawley, R. S. (2003). Chromosome choreography: the meiotic ballet. *Science* **301**, 785-789.
Pasierbek, P., Jantsch, M., Melcher, M., Schleiffer, A., Schweizer, D. and Loidl, J. (2001). A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. *Genes Dev.* **15**, 1349-1360.
Pearlman, R. E., Tsao, N. and Moens, P. B. (1992). Synaptonemal complexes from DNase-treated rat pachytene chromosomes contain (GT)n and LINE/SINE sequences. *Genetics* **130**, 865-872.
Pelttari, J., Hoja, M. R., Yuan, L., Liu, J. G., Brundell, E., Moens, P., Santucci-Darmanin, S., Jessberger, R., Barbero, J. L., Heyting, C. et al. (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., Plug, A. W., van Vugt, M. J. and de Boer, P. (1997). A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res.* **5**, 66-68.
Petronczki, M., Siomos, M. F. and Nasmyth, K. (2003). Un Menage a Quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**, 423-440.
Prieto, I., Suja, J. A., Pezzi, N., Kremer, L., Martinez, A. C., Rufas, J. S. and Barbero, J. L. (2001). Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I. *Nat. Cell Biol.* **3**, 761-766.
Revenkova, E. and Jessberger, R. (2005). Keeping sister chromatids together: cohesins in meiosis. *Reproduction* **130**, 783-790.
Revenkova, E. and Jessberger, R. (2006). Shaping meiotic prophase chromosomes: cohesins and synaptonemal complex proteins. *Chromosoma* **115**, 235-240.
Revenkova, E., Eijpe, M., Heyting, C., Hodges, C. A., Hunt, P. A., Liebe, B., Scherthan, H. and Jessberger, R. (2004). Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. *Nat. Cell Biol.* **6**, 555-562.
Romanienko, P. J. and Camerini-Otero, R. D. (2000). The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol. Cell* **6**, 975-987.
Schleiffer, A., Kaitna, S., Maurer-Stroh, S., Glotzer, M., Nasmyth, K. and Eisenhaber, F. (2003). Kleisins: a superfamily of bacterial and eukaryotic SMC protein partners. *Mol. Cell* **11**, 571-575.
Schwacha, A. and Kleckner, N. (1997). Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell* **90**, 1123-1135.
Shintomi, K. and Hirano, T. (2007). How are cohesin rings opened and closed? *Trends Biochem Sci.* **32**, 154-157.
Siomos, M. F., Badrinath, A., Pasierbek, P., Livingstone, D., White, J., Glotzer, M. and Nasmyth, K. (2001). Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 1825-1835.
Smith, A. V. and Roeder, G. S. (1997). The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J. Cell Biol.* **136**, 957-967.
Song, X., Zhu, C. H., Doan, C. and Xie, T. (2002). Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. *Science* **296**, 1855-1857.
Spradling, A. C. (1993). Germline cysts: communes that work. *Cell* **72**, 649-651.
Stack, S. M. and Anderson, L. K. (2001). A model for chromosome structure during the mitotic and meiotic cell cycles. *Chromosome Res.* **9**, 175-198.
Storlazzi, A., Tesse, S., Gargano, S., James, F., Kleckner, N. and Zickler, D. (2003). Meiotic double-strand breaks at the interface of chromosome movement, chromosome remodeling, and reductional division. *Genes Dev.* **17**, 2675-2687.
Toyoda, Y. and Yanagida, M. (2006). Coordinated requirements of human topo II and cohesin for metaphase centromere alignment under Mad2-dependent spindle checkpoint surveillance. *Mol. Biol. Cell* **17**, 2287-2302.
Valdeolmillos, A., Rufas, J. S., Suja, J. A., Vass, S., Heck, M. M., Martinez, A. C.

and Barbero, J. L. (2004). *Drosophila* cohesins DSA1 and Drad21 persist and colocalize along the centromeric heterochromatin during mitosis. *Biol. Cell* **96**, 457-462.

Valdeolmillos, A. M., Viera, A., Page, J., Prieto, I., Santos, J. L., Parra, M. T., Heck, M. M., Martinez, A. C., Barbero, J. L., Suja, J. A. et al. (2007). Sequential loading of cohesin subunits during the first meiotic prophase of grasshoppers. *PLoS Genet.* **3**, e28.

Vass, S., Cotterill, S., Valdeolmillos, A. M., Barbero, J. L., Lin, E., Warren, W. D. and Heck, M. M. (2003). Depletion of Drad21/Scc1 in *Drosophila* cells leads to instability of the cohesin complex and disruption of mitotic progression. *Curr. Biol.* **13**, 208-218.

Wang, X. and Dai, W. (2005). Shugoshin, a guardian for sister chromatid segregation. *Exp. Cell Res.* **310**, 1-9.

Warren, W. D., Lin, E., Nheu, T. V., Hime, G. R. and McKay, M. J. (2000a). Drad21, a *Drosophila* rad21 homologue expressed in S-phase cells. *Gene* **250**, 77-84.

Warren, W. D., Steffensen, S., Lin, E., Coelho, P., Loupart, M., Cobbe, N., Lee, J. Y., McKay, M. J., Orr-Weaver, T., Heck, M. M. et al. (2000b). The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis. *Curr. Biol.* **10**, 1463-1466.

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

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

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

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