Condensins promote co-orientation of sister chromatids during meiosis I in budding yeast.

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Abstract
The condensin complex is a key determinant of higher-ordered chromosome structure. We show here that the complex is also important for the correct alignment of chromosomes on the meiosis I spindle. Unlike during mitosis and meiosis II, when sister chromatids attach to microtubules emanating from opposite spindle poles (bi-orientation), accurate meiosis I chromosome segregation requires that sister chromatids attach to microtubules emanating from the same spindle pole (co-orientation). The monopolin complex, consisting of Lrs4, Csm1 and the meiosis-specific component Mam1, brings about meiosis I co-orientation. We find that in the absence of functional condensin complexes, a fraction of sister kinetochores bi-orient on the meiosis I spindle and association of the monopolin complex subunit Mam1 with kinetochores is decreased. Our studies uncover a new locus-specific effect of the condensin complex.
Introduction

Meiosis is a cellular division consisting of a single DNA synthesis phase followed by two chromosome segregation phases and is employed in the generation of gametes. During the first meiotic division, homologous chromosomes segregate, requiring that each pair of sister chromatids co-segregate towards one pole (co-orientation); during the second meiotic division, sister chromatids separate towards opposite poles (bi-orientation). In budding yeast, the monopolin complex brings about the co-orientation of sister chromatid kinetochores to allow only one microtubule attachment per pair of sisters (Winey et al., 2005; reviewed in Marston and Amon, 2004). The monopolin complex is composed of four components: Mam1, expressed only during meiosis, which localizes to kinetochores from late pachytene until metaphase I (Toth et al., 2000); Lrs4 and Csm1, two nucleolar components which are released from the nucleolus during prophase I and targeted to kinetochores by the polo-like kinase Cdc5 (Clyne et al., 2003; Lee and Amon, 2003; Rabitsch et al., 2003); and Hrr25, a casein kinase (Petronczki et al., 2006). The monopolin complex is thought to clamp sister kinetochores together through a cohesin-independent mechanism and fuse the two sister kinetochores into a single microtubule attachment site to facilitate co-orientation (Monje-Casas et al., 2007). Deletion of genes encoding monopolin complex subunits results in the bi-orientation of sister chromatids during meiosis I (Toth et al., 2000; Rabitsch et al., 2003; Lee and Amon, 2003; Petronczki et al., 2006).

The condensin complex is a conserved pentameric complex. In budding yeast, it is composed of two coiled-coil SMC (Structural Maintenance of Chromosomes) subunits, Smc2 and Smc4 (Freeman et al., 2000) that form a heterodimer; and a globular head made up of Ycs4 (Bhalla et al., 2002), Ycg1 and Brn1 (Ouspenski et al., 2000). Condensin is best known for its role in chromosomal compaction during mitosis (reviewed in Hirano, 2005) and meiosis (Yu and Koshland, 2003; Chan et al., 2004). During meiosis, the complex is also required for the repair of double strand breaks and chromosome axis morphogenesis, a prerequisite for recombinatorial repair and homolog synapsis as well as resolution of recombination-dependent linkages between homologs (Yu and Koshland, 2003; Yu and Koshland, 2005).

At the rDNA, the site of rRNA synthesis, condensins regulate rDNA silencing and prevent unequal sister chromatid exchange by presumably joining sister chromatid rDNA repeats.
(Freeman et al., 2000; Lavoie et al., 2002; Huang et al., 2006; Waples et al., 2009). Two components of the monopolin complex, Lrs4 and Csm1 share this role with the condensin complex. Both the Lrs4-Csm1 and the condensin complexes reside in the nucleolus (Bhalla et al., 2002; Rabitsch et al., 2003; D’Amours et al., 2004; Huang et al., 2006). There, Lrs4 and Csm1 are part of the RENT (Regulator of nucleolar silencing and telophase exit) complex that binds to the replication fork barrier site within the non-transcribed spacer region NTS1 in the ribosomal DNA repeats (Huang et al., 2006; Waples et al., 2009). Lrs4 and Csm1 regulate rDNA functions by recruiting condensins to the rDNA (Johzuka and Horiuchi, 2009). Condensins and Lrs4-Csm1 not only share functions at the rDNA, their localization at the rDNA appears to be co-regulated. During late anaphase, the Mitotic Exit Network, a signaling pathway that triggers exit from mitosis by promoting the release of the protein phosphatase Cdc14 from the nucleolus, also promotes the dissociation of both the Lrs4-Csm1 complex and condensins from the rDNA (Huang et al., 2006; Varela et al., 2009; I. L. B. unpublished observations).

Lrs4-Csm1 and condensins co-localize at the rDNA where they regulate rDNA stability. During meiosis, Lrs4 and Csm1 associate with kinetochores. Condensins also accumulate at kinetochores in budding (Wang et al., 2004; D’Ambrosio et al., 2008) and fission yeast (Nakazawa et al., 2008). These observations raise the possibility that the two protein complexes also regulate the same process at kinetochores. Our findings lend support to this idea. We find that condensins, like the Lrs4-Csm1 complex, are required for full sister kinetochore co-orientation during meiosis I by promoting the localization of Mam1 to kinetochores. We propose that condensin helps to establish a peri-centromeric architecture required for Mam1 binding.
Materials and Methods

Strains and Growth Conditions
Derivatives of W303 are described in Table 1; derivatives of SK1 strains in Table 2. Proteins were tagged using the PCR-based method described in Longtine et al., 1998. GFP dots were constructed by integrating an array of bacterial TET operator sites 2 kb from the centromere on CENIV in the W303 strains, or 1.4 kb from the centromere of one homolog of chromosome V in the diploid SK1 strains (Toth et al., 2000). Conditions for arrest with α-factor and release from the arrest are as described in Amon, 2002. α-factor was re-added to all cultures 90 minutes after release from the G1 arrest to prevent cells from entering the next cell cycle. Growth conditions for individual experiments are described in the Figure legends.

Sporulation conditions
Cells were grown to saturation in YPD (YEP + 2% glucose) for 24 hours, diluted into YPA (YEP + 2% KAc) at OD₆₀₀ = 0.3 and grown overnight. Cells were then washed with water and resuspended in SPO medium (0.3% KAc [pH = 7.0]) at OD₆₀₀ = 1.9 at 30°C to induce sporulation. Cells carrying temperature sensitive alleles of condensin subunits were induced to sporulate at 25°C for 1 hour and then shifted to 34°C.

Localization techniques
Indirect in situ immunofluorescence was carried out as described in Visintin et al., 1999 for tubulin, HA-, and MYC-tagged proteins. CEN GFP dot visualization was performed as described in (Monje-Casas et al., 2004). Two hundred cells were counted for each time-point. Chromosomes were spread as described in Nairz and Klein, 1997. HA-tagged proteins were detected with a mouse α-HA.11 antibody (Covance) at a 1:500 dilution. MYC-tagged proteins were detected with a mouse anti-MYC 9E10 antibody (Babco) at a 1:500 dilution. Both were followed by a secondary anti-mouse CY3 antibody (Jackson ImmunoResearch) at a 1:1000 dilution. Endogenous luminescence was sufficient for visualizing Ndc80-GFP on chromosome spreads. In spreads of meiotic cells expressing Ndc10-6HA and Mam1-9MYC, mouse anti-HA and rabbit anti-MYC were used at 1:500 dilution, followed by anti-mouse FITC antibodies and
anti-rabbit CY3 antibodies, also used at 1:500 dilution. In each experiment, at least fifty cells were counted per strain.

**Western blot analysis**

Cells were harvested, and incubated in 5% trichloroacetic acid (TCA) and lysed as described in Moll et al., 1991. Immunoblots were performed as described in Cohen-Fix et al., 1996. HA-tagged proteins were detected with a mouse α-HA.11 antibody (Covance) at a 1:500 dilution. MYC-tagged proteins were detected with a mouse anti-MYC 9E10 antibody (Babco) at a 1:1000 dilution. vATPase was detected using a mouse anti-vATPase antibody (Molecular Probes) at a 1:2000 dilution. The secondary antibody used was a goat anti-mouse antibody conjugated to horseradish peroxidase (HRP; Jackson ImmunoResearch) at a 1:2000 dilution. Cdc28 was detected using a rabbit anti-Cdc28 antibody at a 1:1000 dilution. The secondary antibody used was a donkey anti-rabbit antibody conjugated to horseradish peroxidase (HRP; Jackson ImmunoResearch) at a 1:2000 dilution.

**Results**

**Condensins are required for sister kinetochore co-orientation induced by CDC5 and MAM1.**

The observation that the monopolin and condensin complexes function together at the rDNA and are capable of binding to each other (Johzuka and Horiuchi, 2009) prompted us to test the possibility that these proteins function together to maintain genomic integrity at other chromosomal locations. During meiosis, the main function of Lrs4 and Csm1 is to co-orient sister kinetochores (Rabitsch et al., 2003). To determine whether condensins are necessary for this process, we first tested the requirement for the protein complex in a system developed to induce sister kinetochore co-orientation during mitosis. We previously showed that overexpression of the meiosis-specific co-orientation factor Mam1 and the Polo kinase Cdc5 was sufficient to induce sister kinetochore co-orientation during mitosis, leading to co-segregation of sister chromatids during anaphase (Monje-Casas et al., 2007). To follow the segregation of a single pair of sister chromatids, a tandem array of tetO sequences was integrated proximal to the centromere of chromosome IV and a tetR-GFP fusion protein, which binds the tetO sequence, was expressed to visualize the repeats (Michaelis et al., 1997). Overexpression of CDC5 and
MAM1 led to co-segregation of sister chromatids during anaphase in 22% of cells at 34°C (Figure 1A; Monje-Casas et al., 2007). Deletion of LRS4 or CSM1 reduces co-segregation by approximately 50%, whereas deletion of both mitotic components of the monopolin complex almost completely suppressed the co-segregation of sister chromatids induced by high levels of Cdc5 and Mam1 (Figure 1A; Monje-Casas et al., 2007). Inactivation of YCS4 or BRN1 reduced CDC5- and MAM1- induced sister kinetochore co-segregation by approximately 50% (Figure 1A), signifying a role for the condensin complex in establishing sister kinetochore orientation.

The 50% reduction in sister chromatid co-segregation caused by loss of YCS4 and BRN1 function is likely to be an underestimation of the effect of condensin on this process. First, co-segregation of sister chromatids was analyzed only in cells with fully divided nuclei to ensure that these cells had reached anaphase. Cells with stretched or non-divided nuclei, which is indicative of a more complete inactivation of condensin function, were not included in this analysis as co-segregation of sister chromatids cannot be unambiguously determined in such cells. Second, due to low levels of sister chromatid co-segregation already seen in the ycs4-1 and brn1-60 single mutants (Bhalla et al., 2002), the extent of the effects of inactivating YCS4 and BRN1 in reducing CDC5/MAM1-induced sister co-orientation is under-realized (Figure 1A; note that the low-level co-segregation of sister chromatids observed in the condensin single mutants is probably not due to sister kinetochore co-orientation but the result of failed chromosome segregation caused by defects in the decatenation of sister chromatids; Ouspenski et al., 2000; Bhalla et al., 2002). Finally, because the GAL1-10 promoter does not function as well at 37°C compared to 25°C, we performed the experiment at 34°C when the temperature-sensitive condensin alleles may not be completely inactivated. Indeed, at 34°C, both ycs4-1 and brn1-60 mutants exhibited intermediate phenotypes with respect to chromosome segregation during mitosis (Ouspenski et al., 2000; Bhalla et al., 2002). Nevertheless, our results indicate that condensins are required for full sister kinetochore co-orientation induced by Cdc5 and Mam1 overproduction. This loss of co-segregation in the absence of condensin was not due to reduced levels of Cdc5 and Mam1 produced in condensin mutants (Supplemental Figure 1). We conclude that condensin is required for the function of co-orientation factors rather than their production.
To determine how condensins affect sister kinetochore co-orientation, we analyzed the ability of overexpressed Mam1 to associate with kinetochores in condensin mutants by chromosome spreads. In cells overexpressing *CDC5* and *MAM1* during mitosis, Mam1 co-localizes with centromeric GFP dots in approximately 70% of cells with divided nuclei (Figure 1B, C). In contrast, cells carrying the temperature-sensitive condensin allele *ycs4-1* or *brn1-60* exhibited reduced Mam1-9MYC localization to kinetochores. Only 25% of anaphase *ycs4-1* cells and 37% of anaphase *brn1-60* cells were able to target Mam1 to kinetochores at 34°C (Figures 1B, C).

Our results show that condensins are required for full Cdc5/Mam1-induced co-orientation of sister chromatids during mitosis.

**Condensins are required for full sister kinetochore co-orientation during meiosis I.**

Examination of the role of condensin in co-orienting sister chromatids during meiosis I is confounded by its other meiotic functions. Condensin is required during prophase I for processing of double-strand breaks and resolving recombination-dependent chromosome linkages (Yu and Koshland, 2003). To isolate the effect of condensin on co-orientation, we analyzed cells arrested in metaphase I, by depleting the Anaphase Promoting Complex activator Cdc20. In this arrest, sister kinetochores are tightly associated. When the centromere of one homolog is GFP-tagged (heterozygous GFP dots), the pair appears as one focus in the arrest (Lee and Amon, 2003). By contrast, when co-orientation is disrupted, as occurs when *MAM1* is deleted, sister chromatids bi-orient in metaphase I and tension exerted by the meiosis I spindle allows two GFP dots to become visible (Lee and Amon, 2003). To examine the consequences of inactivating condensins on sister kinetochore co-orientation, we analyzed the separation of heterozygous GFP dots in cells carrying temperature-sensitive alleles of *YCG1* and *YCS4*, two genes encoding condensin subunits. Cells were transferred into sporulation-inducing medium; one hour later, they were shifted to 34°C. 60% of Cdc20-depleted cells lacking *MAM1* arrested in metaphase and approximately half of these cells exhibited separated CENV GFP dots (Figure 2; Lee and Amon, 2003). In Cdc20-depleted strains carrying either the *ycg1-2* or *ycs4-2* alleles only 40% of cells reached metaphase, yet, similar to Cdc20-depleted cells lacking *MAM1*, approximately half of the cells showed CENV GFP dot separation (Figure 2). We conclude from this experiment that condensin activity is required to achieve wild-type levels of co-orientation.
during meiosis I. Thus, the condensin complex is required for monopolin complex activity or functions in parallel to the complex to promote sister kinetochore co-orientation.

We also examined sister kinetochore co-orientation in cells depleted of condensins. We generated a depletion allele of the condensin component Brn1 by placing the gene encoding it under the control of the mitosis-specific CLB2 promoter. Epitope-tagged Brn1 produced from the native promoter can be detected throughout meiosis. In contrast, when placed under the CLB2 promoter, Brn1 levels are undetectable after transfer to sporulation medium (Figure 3A). In cells depleted of Brn1, sister kinetochore co-orientation was impaired but the defect was not as severe as that observed in temperature-sensitive condensin mutants. 40% of cells reached metaphase I and 15% showed CENV GFP dot separation (Figure 3B). Similar results were obtained in cells depleted for the condensin subunit Ycs4 (data not shown). The difference in penetrance between temperature-sensitive condensin alleles and the depletion allele could be due to incomplete depletion of Brn1 in CLB2-BRN1 cells and/or due to high temperature exaggerating the co-orientation defect of condensin mutants.

A fraction of cells depleted of Brn1 exhibited stretching of the tetO array. This is evident as lines of tetR-GFP signal (Figure 3C) and has been observed in bi-oriented cells lacking structural integrity at the kinetochores (He et al., 2000; Oliveira et al. 2005; Gerlich et al., 2006; Warsi et al., 2008). These observations suggest a requirement for condensin in sister kinetochore co-orientation and establishing structural integrity at kinetochores during meiosis.

**Condensins are required for Mam1 localization to kinetochores in meiosis I.**

Based on the evidence that condensins were required to localize Mam1 in mitotic cells overexpressing CDC5 and MAM1, we asked whether condensin was also needed for Mam1 association with kinetochores during meiosis I. Mam1 protein accumulation was not affected by the inactivation of condensin (Figure 4A). However, Mam1 association with kinetochores was. Mononucleate cells were viewed 6 hours after transfer into sporulation medium when approximately 30% of wild-type and 20% of Brn1-depleted cells were in metaphase I (Figure 4B). 52% of wild-type cells showed strong co-localization between tagged versions of Mam1 and Ndc10, as expected (Toth et al., 2000). In these cells, the majority of Ndc10 foci (over fifty
percent) were spatially associated with Mam1 foci. Another 27% of cells showed weak co-localization, as defined by a minority of Ndc10 foci displaying Mam1 co-localization (Figures 4C, D). In cells depleted of the condensin component Brn1, only 18% of cells showed Mam1 co-localization with over half of the Ndc10 foci and only an additional 17% showed co-localization with a small fraction (less than 50%) of Ndc10 foci (Figures 4C, D). Results obtained with cells depleted for the condensin component Ycs4 were similar (data not shown). As in cells deleted for LRS4, Mam1 associated with chromatin in Brn1-depleted cells (Supplemental Figure 2, data not shown), but not with kinetochores. We conclude that condensin is required for full association of Mam1 with kinetochores and to bring about co-orientation during meiosis I.

The condensin complex and Lrs4-Csm1 associate with kinetochores through independent mechanisms.

To determine whether the association of the Lrs4-Csm1 complex with meiosis I kinetochores required condensins, we examined Lrs4-6HA localization in chromosome spreads of wild-type and Brn1-depleted cells 6 hours post-transfer into sporulation medium when approximately 20% of cells were in metaphase I (Figure 5A). We were unable to test the localization using temperature-sensitive condensin alleles because they are lethal in the presence of tagged versions of the monopolin complex components (data not shown; Waples et al. 2009.) Lrs4-13MYC was released from the nucleolus (Figure 5A) and co-localized with Ndc10-6HA foci to the same extent in cells depleted for Brn1 as in wild-type cells (Figure 5B, C). In agreement with previous reports that demonstrate that Lrs4 and Csm1 function in a single complex (Rabitsch et al. 2003; Huang et al., 2006), we found Lrs4 and Csm1 localization to be interdependent (Supplemental Figure 3A-C) and Lrs4 protein accumulation to require CSM1 (Supplemental Figure 3D). Our results indicate that condensin is required for Mam1 to associate with kinetochores but not Lrs4 and Csm1, raising the interesting possibility of sequential recruitment of co-orientation factors to kinetochores during meiosis I.

To test whether condensins required the monopolin complex to localize to kinetochores, we used mitotic anaphase-arrested cdc14-3 cells because the congregation of kinetochores during this point of the cell cycle facilitates the analysis of kinetochore proteins (Guacci et al., 1997) and because Lrs4 and Csm1 are enriched at kinetochores in this mutant (I. L. B., unpublished...
observations). We found condensins to be enriched at kinetochores during anaphase (Figure 6), which is consistent with previous results in budding and fission yeast (Wang et al., 2004; Nakazawa et al., 2008; D’Ambrosio et al., 2008). MYC-tagged versions of the condensin subunits Ycs4 (Figures 6A, B) or Smc4 (Figures 6C, D) were found to co-localize with a GFP-tagged version of the kinetochore component Ndc80 in approximately 80% of cells. Deletion of LRS4 (Figure 6A, B) or CSM1 (Figure 6C, D) did not affect this kinetochore association. Together, our findings indicate that although the Lrs4-Csm1 complex is needed for recruitment of the condensin complex to the rDNA (Johzuka and Horiuchi, 2009), the two complexes associate with kinetochores independently of each other.

Discussion

Our studies and those of others indicate that members of the condensin complex and the mitotic components of the monopolin complex, Lrs4 and Csm1, bind at specialized genomic sites where they function to link sister chromatids. We show here that Lrs4-Csm1 and the condensin complex bind to sister kinetochores independently of each other, but that during meiosis, both of these complexes are required to recruit Mam1 (Figure 7A-B). At the rDNA, Lrs4 and Csm1 recruit condensins to inhibit the unequal exchange between sister chromatids (Huang et al., 2006; Johzuka and Horiuchi, 2009), a function most simply explained by a role in linking sister chromatids so that movement of the repeats is restricted with respect to one another (Figure 7C). Together, the two complexes thus provide cohesive properties to sister chromatids and impose steric constraints, to prevent recombination at the rDNA, and at kinetochores, to facilitate their attachment to microtubules emanating from the same spindle pole.

Our results indicate that condensins are required for wild-type levels of association of Mam1 with kinetochores in order to promote the co-orientation of sister chromatids during meiosis I. How could condensin bring this about? It is unlikely that Mam1 fails to associate with kinetochores due to a loss of overall centromeric and pericentromeric structure in condensin mutants. First, kinetochore assembly does not appear to be affected in condensin mutants, as is evident by the sister chromatids’ ability to separate in metaphase I-arrested cells in condensin mutants. This activity requires kinetochores to have captured a microtubule and be under tension. Second, inactivation of the cohesin complex, another SMC chromosome structure complex, does
not affect binding of the monopolin complex to kinetochores (Monje-Casas et al., 2007). Finally, inactivation of condensin does not appear to interfere with the association of Lrs4 with kinetochores. We propose that the condensin complex creates a higher-ordered chromatin structure at the kinetochore, which not only provides a scaffold for the recruitment of Mam1 but that also contributes to the co-orientation process (Figure 7B). Our results also raise the interesting possibility that assembly of the monopolin complex is step-wise, with Lrs4-Csm1 associating with kinetochores independently of Mam1. There they, together with condensins, establish a Mam1 binding platform. We speculate that Lrs4-Csm1 and condensin provide rigidity and hence steric constraints to the centromeric regions and facilitate the linking of microtubule binding sites. This favors orientation of the two sister kinetochores towards one spindle pole. Mam1 could be needed to stabilize these linkages.

Condensins do not appear to be involved in meiosis I sister kinetochore co-orientation in other eukaryotes. In *D. melanogaster* hypomorphic alleles in the gene encoding the non-SMC condensin subunit DCAP-G, (homologous to *S. cerevisiae* Ycg1) do not show defects in co-orientation (Resnick et al., 2009). *C. elegans* mutants in *hcp-6*, a non-SMC condensin II subunit, show defects in chromosome segregation during meiosis I (Chan et al., 2004) but whether sister kinetochore co-orientation is affected in this mutant is not clear. It appears that in most species, the other SMC-containing chromosome structure complex, the cohesin complex, facilitates sister kinetochore co-orientation. In fission yeast, meiotic cohesin complexes associate with the core centromere where they, together with the co-orientation factor Moa1, facilitate a kinetochore geometry that favors sister kinetochore co-orientation (Yokobayashi and Watanabe, 2005; Sakuno et al., 2009). In maize and Arabidopsis, cohesins are also essential for sister kinetochore co-orientation (Yu and Dawe, 2000; Chelysheva et al., 2005). Clearly, SMC protein-containing complexes play critical roles in establishing co-orientation but a role for condensin has thus far only been conclusively demonstrated in yeast. We speculate that employing different SMC complexes in promoting sister kinetochore co-orientation reflects differences in kinetochore architecture. Organisms in which sister kinetochore co-orientation requires cohesins have regional centromeres with large pericentromeres that are heterochromatic in nature. In contrast, budding yeast centromeres are only 147bp in length and lack pericentromeric heterochromatin. Structural rigidity appears necessary for sister kinetochore co-orientation (Sakuno et al., 2009).
At centromeres surrounded by heterochromatin, cohesin could be sufficient to bring about this rigidity. At centromeres that lack a heterochromatic pericentromere, additional rigidity factors such as condensins could be important. Consistent with a role of condensins in providing chromatin firmness in the pericentromere is our observation that tetO arrays located next to the centromere appear stretched in condensin mutants. By structuring peri-centromeric regions and linking them to centromeric proteins, namely Lrs4 and Csm1, condensins could promote sister kinetochore co-orientation.

Our data further add to the growing body of evidence to suggest that condensins not only establish gross chromosomal architecture (reviewed in Hirano, 2005) but also play a role in creating locus-specific chromosome structures. At the rDNA, condensin binds distinct rDNA repeats to prevent recombination between mismatched rDNA repeats (Huang et al. 2006; Johzuka and Horiuchi, 2009). Clustering of tRNA genes by condensin is used to modulate their expression levels (Haesler et al., 2008; D’Ambrosio et al., 2008). Our data suggest that condensin plays a role in co-orientation at centromeric regions. The generation of specialized local chromatin structure may utilize condensin’s ability to aggregate DNA in a locus-specific manner.

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Figures

Figure 1. Condensin is required for sister kinetochore co-orientation induced by high levels of Mam1 and Cdc5.

(A) Wild-type (A5244), pGAL-CDC5 pGAL-MAM1 (A12312), pGAL-CDC5 pGAL-MAM1 lrs4Δ (A15910), pGAL-CDC5 pGAL-MAM1 lrs4Δ csm1Δ (A21128), pGAL-CDC5 pGAL-MAM1 ycs4-1 (A20739), ycs4-1 (A21818), pGAL-CDC5 pGAL-MAM1 brn1-60 (A21712), and brn1-60 cells (A21688), all carrying CENIV GFP dots, were arrested in G1 using 5 μg/ml α-factor in YEP medium containing 2% raffinose. One hour prior to release, galactose (2%) was added to induce MAM1 and CDC5 expression. Cells were released into YEP medium containing 2% raffinose and 2% galactose at 34°C. The percentage of anaphase cells in which GFP dots co-segregated (dark grey bars) was determined. 200 cells were counted per strain. Bars represent standard deviation. Statistical significance was measured using a single-factor ANOVA test. p-values are represented by the asterisks (p<0.05= * ; p<0.01= **; p<0.001= ***). (B-C) pGAL-CDC5 pGAL-MAM1 (A12312), pGAL-CDC5 pGAL-MAM1 ycs4-1 (A20739), and pGAL-CDC5 pGAL-MAM1 brn1-60 (A21712) were grown as described in (A) to determine the co-localization of Mam1-9MYC with both, one or neither CENIV GFP dots by chromosome spreads of anaphase cells (B). The micrographs in (C) show Mam1-9MYC (red) and CENIV-GFP (green) localization. At least 50 cells were counted per strain.

Figure 2. Condensin is required for sister kinetochore co-orientation during meiosis I.

pCLB2-CDC20 (A7118, diamonds), pCLB2-CDC20 mam1Δ (A7316, squares), pCLB2-CDC20 ycg1-2 (A23218, triangles), and pCLB2-CDC20 ycs4-2 (A23220, circles) cells containing heterozygous CENV GFP dots were induced to sporulate at 25°C. One hour after transfer into sporulation medium, cells were shifted to 34°C. At the indicated times, samples were taken to determine the percentage of cells with metaphase I spindles (left graph) and separated CENV GFP dots (right graph). 200 cells were counted per time-point.

Figure 3. Condensin is required to maintain peri-centromeric structure.

(A) 3HA-BRN1 (HY1143) and pCLB2-3HA-BRN1 (3069C) cells were induced to sporulate and 3HA-Brn1 protein levels were examined in wild-type (left) and pCLB2-3HA-BRN1 (right) cells.
β-tubulin was used as a loading control. (B, C) pCLB2-CDC20 (A7118, diamonds), pCLB2-CDC20 mam1Δ (A7316, squares), pCLB2-CDC20 pCLB2-BRN1 (A22520, triangles) diploid cells containing heterozygous CENV GFP dots were induced to sporulate at 30°C. At the indicated times, samples were taken to determine the percentage of cells with metaphase I spindles (B, left graph) and separated CENV GFP dots (B, right graph). 10 hours after transfer into sporulation medium, the percentages of cells displaying stretched CENV GFP signal was determined (C). 200 cells were counted per time-point.

Figure 4. Condensins are required for Mam1 localization to kinetochores.
(A-B) Wild-type (A7097, circles) and pCLB2-3HA-BRN1 (A22517, squares) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°C. At the indicated times, samples were taken to determine the levels of Mam1-9MYC (A). Cdc28 was used as a loading control. Samples were also taken to determine the percentage of cells with metaphase I spindles (B). 200 cells were counted per time-point. (C, D) Wild-type (A7097) and pCLB2-3HA-BRN1 (A22517) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°C. Chromosome spreads were performed on cells 6 hours after transfer into sporulation medium. The percentage of cells showing over 50%, less than 50% or no co-localization between Mam1-9MYC (red) and Ndc10-6HA (green) foci was determined (C). The micrographs in (D) show examples of Mam1 association with kinetochores (top panels), taken from wild-type cells, and Mam1 association with chromosomes but not kinetochores (bottom panels) taken from Brn1-depleted cells. At least 50 cells were counted per strain.

Figure 5. Lrs4 localizes to kinetochores independently of the condensin complex.
Wild-type (A9043) and pCLB2-3HA-BRN1 (A23861) cells carrying Lrs4-13MYC and Ndc10-6HA fusions were induced to sporulate at 30°C. At the indicated times, samples were taken to determine the percentage of cells with metaphase I (diamonds) and anaphase I spindles (squares) and the percentage of cells with Lrs4-13MYC released from the nucleolus (open circles; A). Chromosome spreads were performed on cells 6 hours after transfer into sporulation medium. The percentage of cells showing over 50%, less than 50% or no co-localization of the Lrs4-13MYC (green) and Ndc10-6HA (red) foci was determined (B). The micrographs in (C) show
examples of Lrs4 association with kinetochores taken from wild-type cells (top panels) and Brn1-depleted cells (bottom panels). At least 50 cells were counted per strain.

**Figure 6. Condensin association with kinetochores is independent of Lrs4-Csm1.**

(A-B) *cdc14-3* (A20336) and *cdc14-3 lrs4Δ* (A21607) cells carrying Ndc80-GFP and Ycs4-13MYC fusions were released from a pheromone-induced G1 arrest at 37°C. Chromosome spreads were performed on samples taken 150 minutes after release to determine the percentage of cells showing strong, weak or no co-localization of Ycs4-13MYC with Ndc80-GFP (A). Strong co-localization refers to Ycs4 staining at Ndc80 foci that is at or above the level of Ycs4 staining at the rDNA. Weak co-localization refers to Ycs4 signal at either only one Ndc80 focus or Ycs4 signal at both Ndc80 foci that is weaker than Ycs4 signal at the rDNA. The micrographs in (B) show Ycs4-13MYC (red) and Ndc80-GFP (green) localization in *cdc14-3* and *cdc14-3 lrs4Δ* mutants. At least 50 cells were counted per strain. (C-D) *cdc14-3* (A21860) and *cdc14-3 csm1Δ* (A21861) cells carrying Ndc80-GFP and Smc4-13MYC fusions were grown and processed as described in (A) to determine the percentage of cells showing strong, weak or no co-localization of Smc4-13MYC with Ndc80-GFP (C). Categories are described in the legend to figure (A, B). The micrographs in (D) show Smc4-13MYC (red) and Ndc80-GFP (green) localization in *cdc14-3* and *cdc14-3 csm1Δ* mutants. At least 50 cells were counted per strain.

**Figure 7. A speculative model for how condensins and Lrs-Csm1 promote sister kinetochore co-orientation and prevent unequal recombination of rDNA repeats.**

Our data suggest a model where Lrs4 and Csm1 collaborate with condensins to bring about the co-orientation of sister chromatids during meiosis I. Lrs4-Csm1 and condensins independently associate with kinetochores and peri-centromeric regions, respectively (A). We speculate that once recruited, Lrs4 and Csm1 aggregate condensin complexes, thereby physically constraining the two kinetochores. This permits the association of Mam1, which promotes sister kinetochore attachment to microtubules emanating from the same pole (B). Lrs4-Csm1 function at kinetochores is analogous to their role within the nucleolus. There, the recruitment hierarchy is different, with Fob1 and other RENT complex components recruiting Lrs4-Csm1. These two proteins in turn, bind to condensins and recruit them to the rDNA, where they bring about higher-order chromosome structure. Lrs4-Csm1 “zip up” condensin complexes or restrict their
movement with respect to each other, thereby preventing the interaction of rDNA repeats with repeats that are not at the homologous position (C). We suggest that Lrs4-Csm1 collaborate with condensins to create higher-order chromosomal structures.
Supplemental Figures

Supplemental Figure 1. Cdc5 and Mam1 induction from the GAL1-10 promoter in ycs4-1 or brn1-60 cells.
Exponentially growing wild-type (A12312), ycs4-1 (A20739) and brn1-60 (A21712) cells carrying pGAL-3HA-MAM1 and pGAL-3MYC-CDC5 fusions were treated with galactose for 1 hour at 25°C and then shifted to 34°C. Samples were taken after 90 minutes to determine the levels of Cdc5 and Mam1 protein. Cdc28 was used as a loading control.

Supplemental Figure 2. Lrs4 is not required for Mam1 association with chromatin.
Wild-type (A7097) and lrs4Δ (A24443) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°C. At the indicated times, samples were taken to determine the percent of cells in metaphase I (diamonds) or anaphase I (squares) (A). 200 cells were counted per time-point. Spreads were performed at 6 and 7 hours to determine the number of mono-nucleates with Mam1-9MYC bound to chromatin (B). This study revealed that although Mam1 does not associate with kinetochores in lrs4Δ cells (Rabitsch et al, 2003) it associates with chromatin. At least 50 cells were counted per strain.

Supplemental Figure 3. The localization of Lrs4 and Csm1 is interdependent.
(A-B) Wild-type (A13838; A) and csm1Δ (A15974; B) cells carrying an Lrs4-6HA fusion were released from a pheromone-induced G1 arrest at 25°C. At the indicated times, samples were taken to determine the percentage of cells with metaphase (diamonds) and anaphase spindles (squares) and the percentage of cells showing the release of Lrs4-6HA from the nucleolus (open circles). 200 cells were counted per time-point. In csm1Δ cells, Lrs4 staining is diffuse throughout the cell and was not scored as a normal anaphase release. (C) The micrographs show examples of the localization of Csm1-9MYC (red) and tubulin (green) in wild-type cells (A15087) (top panels) and lrs4Δ cells (A15976) (bottom panels). DNA is shown in blue. The uppermost row shows nucleolar sequestration during G1, whereas the second row shows nuclear release during anaphase. (D) Left: Levels of Lrs4-6HA in exponentially growing wild-type (A13838) and csm1Δ (A15974) cells at 25°C. Right: Levels of Csm1-9MYC in exponentially
growing wild-type (A15087) and \(lrs4\Delta\) (A15976) cells at 25ºC. vATPase was used as a loading control.

**Supplemental Table 1. Strain derivatives of W303 used in this study**

Note: All strains are derivatives of W303 and share the same markers as A2587 unless otherwise noted.

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**Supplemental Table 2. Strain derivatives of SK1 used in this study**

Note: All strains are derivatives of SK1 and share the same markers as A4962 unless otherwise noted.

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<td>HY3069C</td>
<td>MATa/α, *ura3/ura3, leu2/leu2, brn1::pCLB2-3HA-BRN1::Kan/brn1::pCLB2-3HA-BRN1::Kan</td>
</tr>
</tbody>
</table>
FIGURE 1

A

Percent anaphase cells

WT   pGAL-CDC5   pGAL-MAM1  brn1-60
pGAL-CDC5   pGAL-MAM1  brn1-60
pGAL-CDC5   pGAL-MAM1  brn1-60
pGAL-CDC5   pGAL-MAM1  brn1-60

B

Percent anaphase cells

pGAL-CDC5   pGAL-MAM1  ycs4-1
pGAL-CDC5   pGAL-MAM1  ycs4-1
pGAL-CDC5   pGAL-MAM1  ycs4-1
pGAL-CDC5   pGAL-MAM1  ycs4-1

Colocalization with both CENIV foci
Colocalization with one CENIV focus
Colocalization with neither CENIV foci

C

DAPI  CENIV  Mam1-9MYC  Merge
pGAL-CDC5  pGAL-MAM1
pGAL-CDC5  pGAL-MAM1  ycs4-1
pGAL-CDC5  pGAL-MAM1  ycs4-1
pGAL-CDC5  pGAL-MAM1  brn1-60

pGAL-CDC5  pGAL-MAM1  brn1-60
pGAL-CDC5  pGAL-MAM1  brn1-60
FIGURE 2

[Graph showing the percentage of metaphase cells and the percent separated CEN dots over time for different strains: pCLB2-CDC20, pCLB2-CDC20 mam1Δ, pCLB2-CDC20 yg1-2, pCLB2-CDC20 ycs4-2.]

[Logarithmic scale for percent metaphase cells, 0 to 100, and linear scale for percent separated CEN dots, 0 to 40.]
FIGURE 3

A

3HA-BRN1

β-tub

$$\begin{array}{c}
\text{HA} \\
\text{β-tub}
\end{array}$$

$$\begin{array}{c}
\text{3HA-BRN1} \\
\text{pCLB2-3HA-BRN1}
\end{array}$$

Hours after induction of meiosis

B

Percent metaphase cells

Percent separated CENV dots

Percent bioriented cells with stretched CENV

Graphs showing the percentage of cells at different stages over time.

C

Bar graph showing the percentage of cells with separated and bioriented CENV dots.

Legend:

- pCLB2-CDC20
- pCLB2-CDC20 mam1Δ
- pCLB2-CDC20 pCLB2-BRN1
FIGURE 4

A

B

C

D

MAM1-9MYC NDC10-6HA

pCLB2-3HA-BRN1

MAM1-9MYC NDC10-6HA

MAM1-9MYC NDC10-6HA

pCLB2-3HA-BRN1

Percent metaphase I cells

Percent cells at 6 hours

DAPI            Ndc10-6HA     Mam1-9MYC        Merge

(Mislocalized)    (Localized)

Wild-type

pCLB2-3HA-BRN1

Mam1 colocalization with >50% Ndc10 foci
Colocalization with < 50% of Ndc10 foci
No Mam1 colocalization with Ndc10 foci

Time (hours)
**FIGURE 5**

A

LRS4-13MYC NDC10-6HA

- metaphase I
- anaphase I
- Lrs4 released

B

Percent cells

Time (hours)

LRS4-13MYC NDC10-6HA

- metaphase I
- anaphase I
- Lrs4 released

pCLB2-3HA-BRN1

C

DAPI Ndc10-6HA Lrs4-13MYC Merge

Wildtype

pCLB2-3HA-BRN1
Percent anaphase cells

YCS4-13MYC NDC80-GFP

CDC14-3 CDC14-3 LRS4Δ

Strong colocalization with both Ndc80 foci
Weak colocalization with both Ndc80 foci,
or colocalization with only one Ndc80 focus
Colocalization with neither Ndc80 foci

SMC4-13MYC NDC80-GFP

CDC14-3 CDC14-3 CSM1Δ

Strong colocalization with both Ndc80 foci
Weak colocalization with both Ndc80 foci,
or colocalization with only one Ndc80 focus
Colocalization with neither Ndc80 foci

FIGURE 6

A       B

C       D

DAPI   Ndc80-GFP   Ycs4-MYC   Merge

dcc14-3

dcc14-3

DAPI   Ndc80-GFP   Ycs4-MYC   Merge

dcc14-3

dcc14-3

Strong colocalization with both Ndc80 foci
Weak colocalization with both Ndc80 foci,
or colocalization with only one Ndc80 focus
Colocalization with neither Ndc80 foci
FIGURE 7

A  B  C

At kinetochores

At the rDNA

Diagram showing interactions between Condensin, Kinetochore, Centromere, Lrs4/Csm1, Mam1/Hrr25, and NTS1/RFB in different contexts.