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Condensins Promote Coorientation 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 (biorientation), accurate meiosis I chromosome segregation requires that sister chromatids attach to microtubules emanating from the same spindle pole (coorientation). The monopolin complex, consisting of Lrs4, Csm1, and the meiosis-specific component Mam1, brings about meiosis I coorientation. We find that in the absence of functional condensin complexes, a fraction of sister kinetochores biorient 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.

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 cosegregates toward one pole (coorientation); during the second meiotic division, sister chromatids separate toward opposite poles (biorientation). In budding yeast, the monopolin complex brings about the coorientation 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 that 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 coorientation (Monje-Casas *et al.* 2007). Deletion of genes encoding monopolin complex subunits results in the biorientation of sister chromatids during meiosis I (Toth *et al.* 2000; Lee and Amon 2003; Rabitsch *et al.* 2003; Petronczki *et al.* 2006).

The condensin complex is a conserved pentameric complex. In budding yeast, it is composed of two coiled-coil structural maintenance of chromosomes (SMC) 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, 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 **re**gulator of **n**ucleolar silencing and **t**elophase exit (RENT) complex that binds to the replication fork barrier site within the non-transcribed spacer region NTS1 in the ribosomal DNA

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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). Not only do condensins and Lrs4-Csm1 share functions at the rDNA, but also their localization at the rDNA appears to be coregulated. 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. Brito, unpublished observations).

Lrs4-Csm1 and condensins colocalize 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 coorientation during meiosis I by promoting the localization of Mam1 to kinetochores. We propose that condensin helps to establish a pericentromeric architecture required for Mam1 binding.

MATERIALS AND METHODS

Strains and growth conditions: Derivatives of W303 are described in supporting information, Table S1; derivatives of SK1 strains are in Table S2. 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 readded to all cultures 90 min after release from the G_1 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 YEP + 2% glucose (YPD) for 24 hr, diluted into YEP + 2% KAc (YPA) at $OD_{600} = 0.3$, and grown overnight. Cells were then washed with water and resuspended in SPO medium [0.3% KAc (pH = 7.0)] at $OD_{600} = 1.9$ at 30° to induce sporulation. Cells carrying temperature-sensitive alleles of condensin subunits were induced to sporulate at 25° for 1 hr and then shifted to 34°.

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.* 2007). 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 50 cells were counted per strain.

Western blot analysis: Cells were harvested, 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, Eugene, OR) 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 HRP (Jackson ImmunoResearch) at a 1:2000 dilution.

RESULTS

Condensins are required for sister kinetochore coorientation 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 coorient 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 coorientation during mitosis. We previously showed that overexpression of the meiosis-specific coorientation factor Mam1 and the Polo kinase Cdc5 was sufficient to induce sister kinetochore coorientation during mitosis, leading to cosegregation 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 cosegregation of sister chromatids during anaphase in 22% of cells at 34° (Figure 1A) (Monje-Casas *et al.* 2007). Deletion of *LRS4* or *CSM1* reduces cosegregation by ~50%, whereas deletion of both mitotic components of the monopolin complex almost completely suppressed the cosegregation 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 cosegregation by ~50% (Figure 1A), signifying a role for the condensin complex in establishing sister kinetochore orientation.

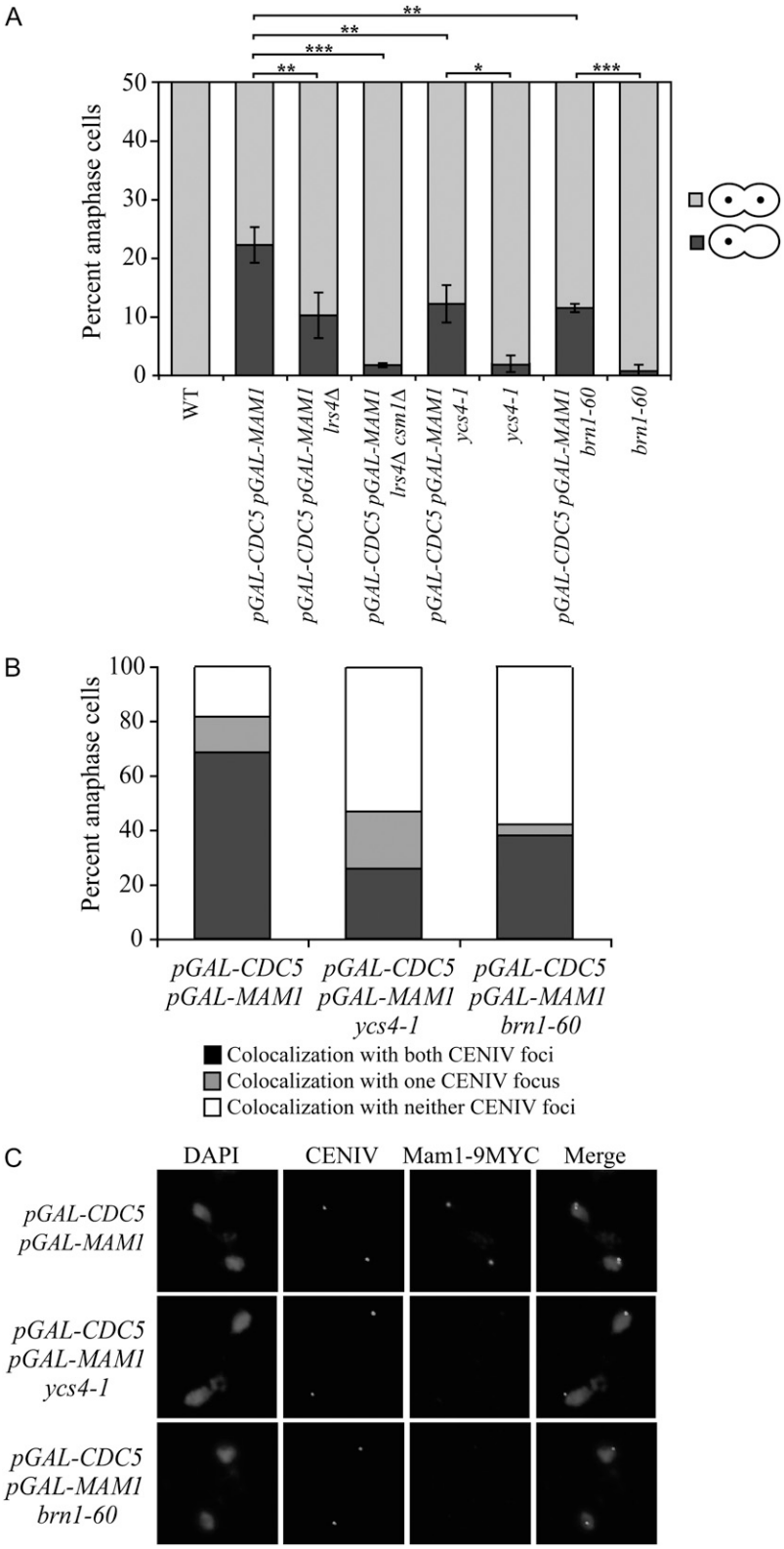

FIGURE 1.—Condensin is required for sister kinetochore coorientation induced by high levels of Mam1 and Cdc5. (A) Wild-type (A5244), *pGAL-CDC5 pGAL-MAM1* (A12312), *pGAL-CDC5 pGAL-MAM1 lrs4*Δ (A15910), *pGAL-CDC5 pGAL-MAM1 lrs4*Δ *csm1*Δ (A21128), *pGAL-CDC5 pGAL-MAM1 ycs4-1* (A20739), *ycs4-1* (A21818), *pGAL-CDC5 pGAL-MAM1 brn1-60* (A21712), and *brn1-60* cells (A21688), all carrying CENIV GFP dots, were arrested in G_1 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°. The percentage of anaphase cells in which GFP dots cosegregated (dark gray bars) was determined. Two hundred 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 and 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 colocalization of Mam1-9MYC with both, one, or no 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.

The 50% reduction in sister chromatid cosegregation caused by loss of *YCS4* and *BRN1* function is likely to be an underestimation of the effect of condensin on this process. First, cosegregation of sister chromatids was analyzed only in cells with fully divided nuclei to ensure that these cells had reached anaphase. Cells with stretched or nondivided nuclei, which is indicative of a more complete inactivation of condensin function, were not included in this analysis as cosegregation of sister chromatids cannot be unambiguously determined in such cells. Second, due to low levels of sister chromatid cosegregation already seen in the *ycs4-1* and *brn1-60*

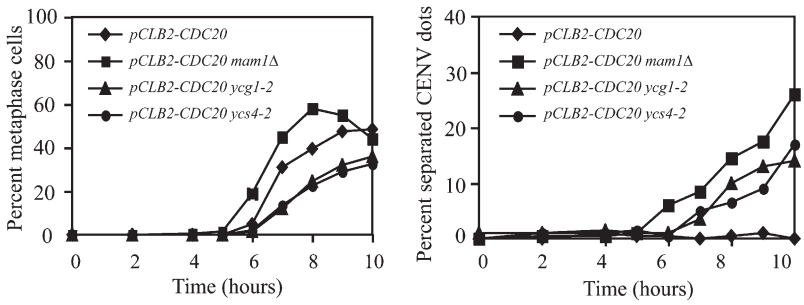

FIGURE 2.—Condensin is required for sister kinetochore coorientation during meiosis I. pCLB2-CDC20 (A7118, diamonds), pCLB2-CDC20 mam1Δ (A7316, squares), pCLB2-CDC20 ycg1-2 (A23218, triangles), and pCLB2-CDC20 ycs4-2 (A23220, circles) cells containing heterozygous CENV GFP dots were induced to sporulate at 25°. One hour after transfer into sporulation medium, cells were shifted to 34°. 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). Two hundred cells were counted per time point.

single mutants (BHALLA *et al.* 2002), the extent of the effects of inactivating *YCS4* and *BRN1* in reducing Cdc5/Mam1-induced sister coorientation is underrealized (Figure 1A; note that the low-level cosegregation of sister chromatids observed in the condensin single mutants is probably not due to sister kinetochore coorientation 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° compared to 25°, we performed the experiment at 34° when the temperature-sensitive condensin alleles may not be completely inactivated. Indeed, at 34°, 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 coorientation induced by Cdc5 and Mam1 overproduction. This loss of cosegregation in the absence of condensin was not due to reduced levels of Cdc5 and Mam1 produced in condensin mutants (Figure S1). We conclude that condensin is required for the function of coorientation factors rather than their production.

To determine how condensins affect sister kinetochore coorientation, 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 colocalizes with centromeric GFP dots in ~70% of cells with divided nuclei (Figure 1, B and 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° (Figure 1, B and C). Our results show that condensins are required for full Cdc5/Mam1-induced coorientation of sister chromatids during mitosis.

Condensins are required for full sister kinetochore coorientation during meiosis I: Examination of the role

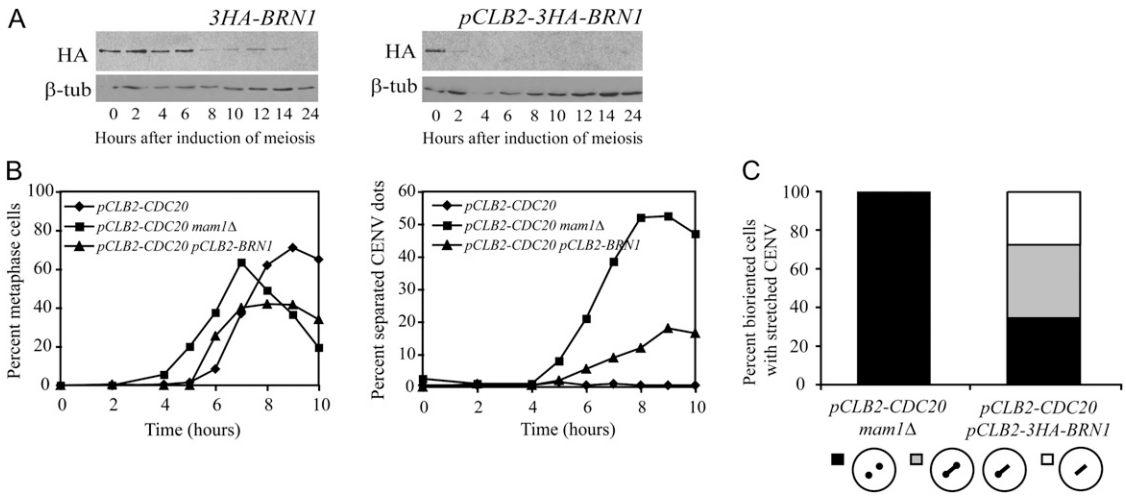

FIGURE 3.—Condensin is required to maintain pericentromeric structure. (A) *3HA-BRN1* (HY1143) and *pCLB2-3HA-BRN1* (3069C) cells were induced to sporulate and 3HA-Brn1 protein levels were examined in wild-type (left) and *pCLB2-3HA-BRN1* (right) cells. β-Tubulin was used as a loading control. (B and C) *pCLB2-CDC20* (A7118, diamonds), *pCLB2-CDC20 mam1Δ* (A7316, squares), and *pCLB2-CDC20 pCLB2-BRN1* (A22520, triangles) diploid cells containing heterozygous CENV GFP dots were induced to sporulate at 30°. 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). (C) Ten hours after transfer into sporulation medium, the percentage of cells displaying stretched CENV GFP signal was determined. Two hundred cells were counted per time point.

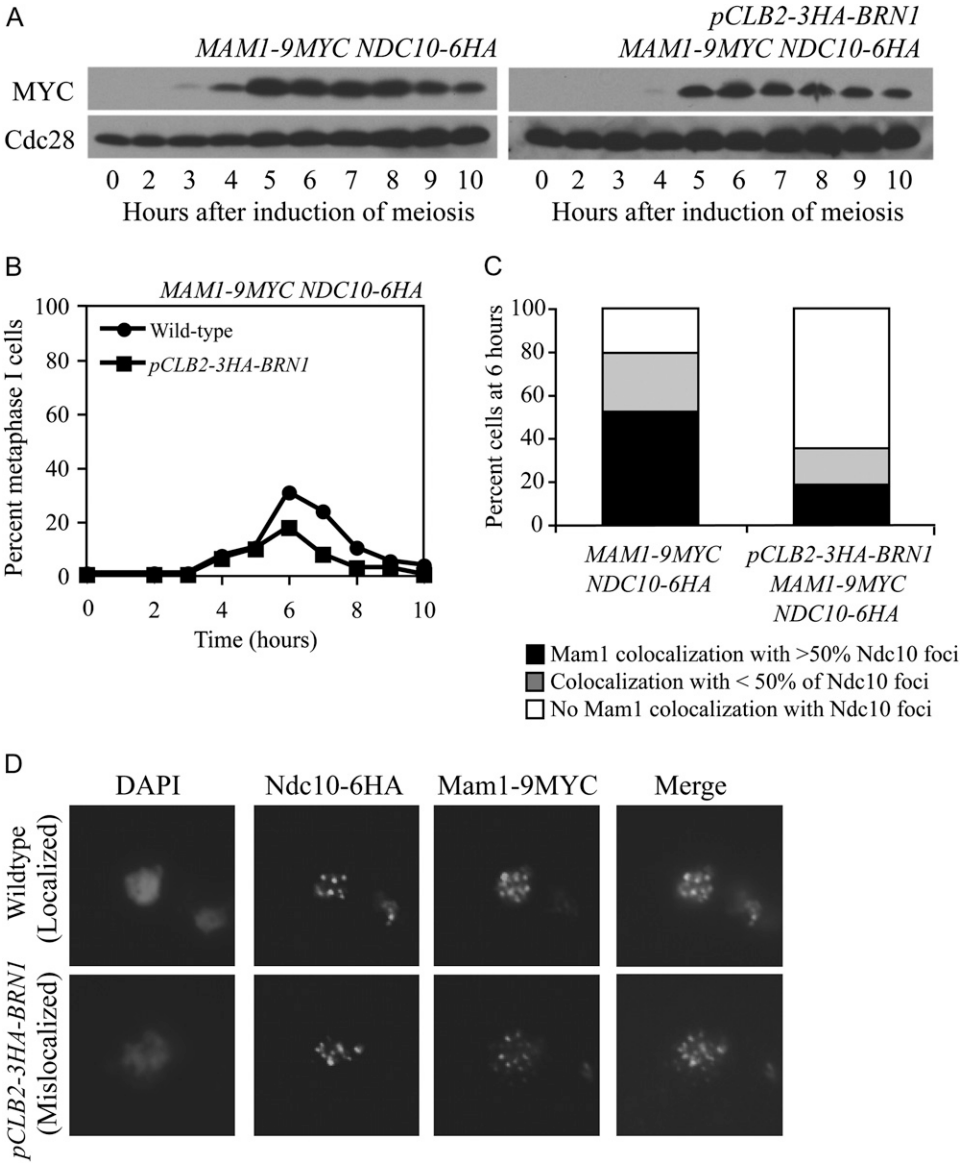

FIGURE 4.—Condensins are required for Mam1 localization to kinetochores. (A and B) Wild-type (A7097, circles) and *pCLB2-3HA-BRN1* (A22517, squares) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°. (A) At the indicated times, samples were taken to determine the levels of Mam1-9MYC. Cdc28 was used as a loading control. (B) Samples were also taken to determine the percentage of cells with metaphase I spindles. Two hundred cells were counted per time point. (C and D) Wild-type (A7097) and *pCLB2-3HA-BRN1* (A22517) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°. Chromosome spreads were performed on cells 6 hr after transfer into sporulation medium. (C) The percentage of cells showing >50%, <50%, or no colocalization between Mam1-9MYC (red) and Ndc10-6HA (green) foci was determined. 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.

of condensin in coorienting 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 coorientation, 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 coorientation is disrupted, as occurs when *MAM1* is deleted, sister chromatids biorient 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 coorientation, 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; 1 hr later, they were shifted to 34°. Sixty percent 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 the *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 coorientation during meiosis I. Thus, the condensin complex is required for monopolin complex activity or functions in parallel to the complex to promote sister kinetochore coorientation.

We also examined sister kinetochore coorientation in cells depleted of condensins. We generated a depletion

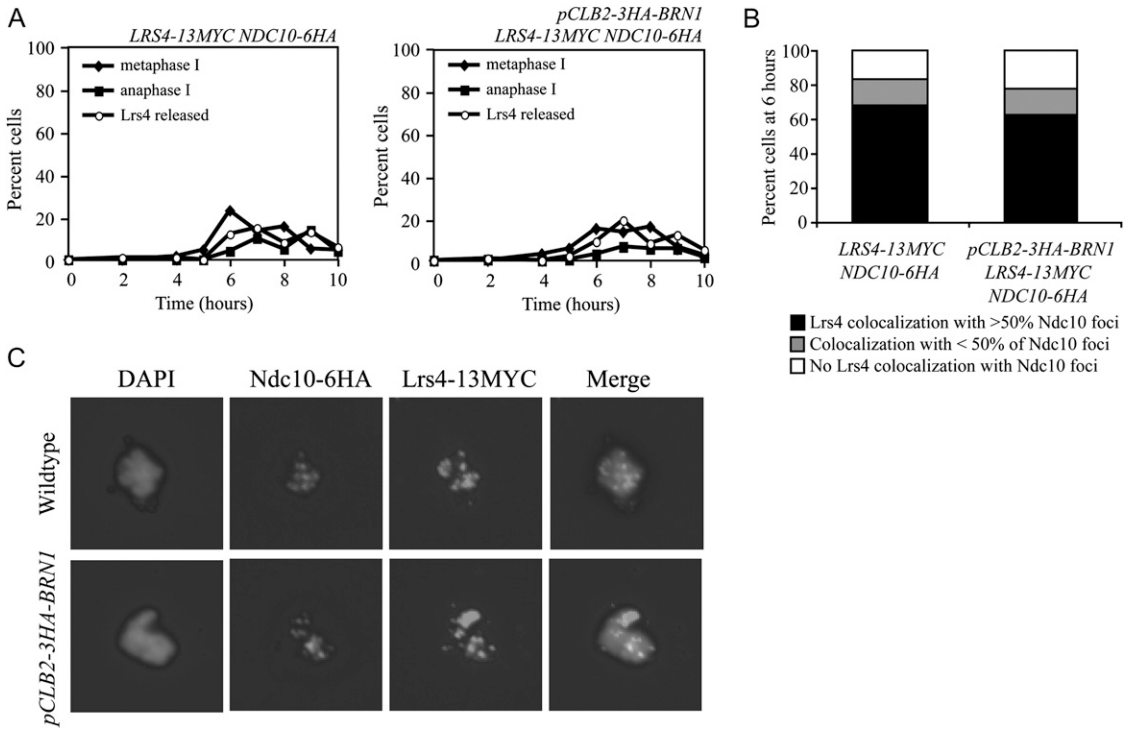

FIGURE 5.—Lrs4 localizes to kinetochores independently of the condensin complex. Wild-type (A9043) and *pCLB2-3HA-BRN1* (A23861) cells carrying Lrs4-13MYC and Ndc10-6HA fusions were induced to sporulate at 30°. (A) At the indicated times, samples were taken to determine the percentage of cells with metaphase I (diamonds) and anaphase I (squares) spindles and the percentage of cells with Lrs4-13MYC released from the nucleolus (open circles). Chromosome spreads were performed on cells 6 hr after transfer into sporulation medium. (B) The percentage of cells showing >50%, <50%, or no colocalization of the Lrs4-13MYC (green) and Ndc10-6HA (red) foci was determined. 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.

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 coorientation was impaired but the defect was not as severe as that observed in temperature-sensitive condensin mutants. Forty percent of cells reached metaphase I and 15% showed CENV GFP dot separation (Figure 3B). Similar results were obtained in cells depleted of 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 coorientation 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 bioriented 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 coorientation and in establishing structural integrity at kinetochores during meiosis.

Condensins are required for Mam1 localization to kinetochores in meiosis I: On the basis of 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 hr after transfer into sporulation medium when ~30% of wild-type and 20% of Brn1-depleted cells were in metaphase I (Figure 4B). Fifty-two percent of wild-type cells showed strong colocalization between tagged versions of Mam1 and Ndc10, as expected (Toth *et al.* 2000). In these cells, the majority of Ndc10 foci (>50%) were spatially associated with Mam1 foci. Another 27% of cells showed weak colocalization, as defined by a minority of Ndc10 foci displaying Mam1 colocalization (Figure 4, C and D). In cells depleted of the condensin component Brn1, only 18% of cells showed Mam1 colocalization with over half of the Ndc10 foci and only an additional 17% showed colocalization with a small

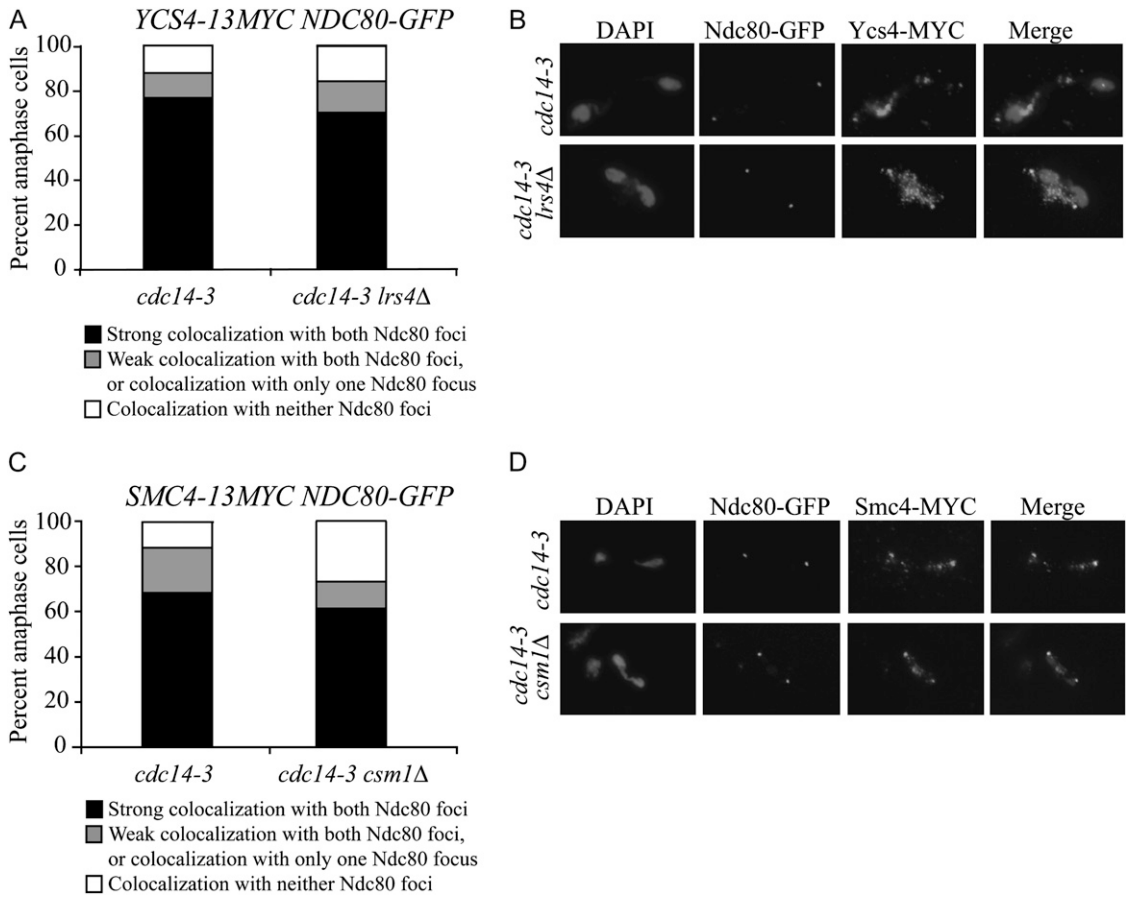

Figure 6.—Condensin association with kinetochores is independent of Lrs4-Csm1. (A and B) *cdc14-3* (A20336) and *cdc14-3 lrs4*Δ (A21607) cells carrying Ndc80-GFP and Ycs4-13MYC fusions were released from a pheromone-induced G_1 arrest at 37°. (A) Chromosome spreads were performed on samples taken 150 min after release to determine the percentage of cells showing strong, weak, or no colocalization of Ycs4-13MYC with Ndc80-GFP. Strong colocalization refers to Ycs4 staining at Ndc80 foci that is at or above the level of Ycs4 staining at the rDNA. Weak colocalization refers either to Ycs4 signal at 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 lrs4*Δ mutants. At least 50 cells were counted per strain. (C and D) *cdc14-3* (A21860) and *cdc14-3 csm1*Δ (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 colocalization of Smc4-13MYC with Ndc80-GFP. (C) Categories are described as in A and B. The micrographs in D show Smc4-13MYC (red) and Ndc80-GFP (green) localization in *cdc14-3* and *cdc14-3 csm1*Δ mutants. At least 50 cells were counted per strain.

fraction (<50%) of Ndc10 foci (Figure 4, C and D). Results obtained with cells depleted of the condensin component Ycs4 were similar (data not shown). As in cells lacking *LRS4*, Mam1 associated with chromatin in Brn1-depleted cells (Figure S2, data not shown), but not with kinetochores. We conclude that condensin is required for full association of Mam1 with kinetochores and to bring about coorientation 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 hr post-transfer into sporulation medium when ~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 colocalized with Ndc10-6HA foci to the same extent in cells depleted for Brn1 as in wild-type cells (Figure 5, B and 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 (Figure S3, A–C) and Lrs4 protein accumulation to require *CSM1* (Figure S3 D). 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 coorientation factors to kinetochores during meiosis I.

To test whether condensins required the monopolin complex to localize to kinetochores, we used mitotic

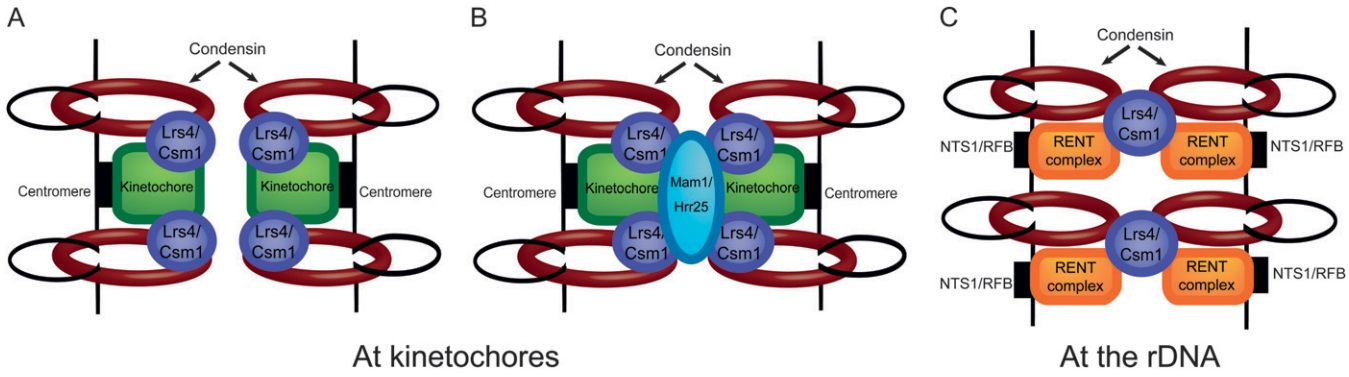

Figure 7.—A speculative model for how condensins and Lrs-Csm1 promote sister kinetochore coorientation and prevent unequal recombination of rDNA repeats. Our data suggest a model where Lrs4 and Csm1 collaborate with condensins to bring about the coorientation of sister chromatids during meiosis I. Lrs4-Csm1 and condensins independently associate with kinetochores and pericentromeric 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.

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. Brito, 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; D'Ambrosio *et al.* 2008; Nakazawa *et al.* 2008). MYC-tagged versions of the condensin subunits Ycs4 (Figure 6, A and B) or Smc4 (Figure 6, C and D) were found to colocalize with a GFP-tagged version of the kinetochore component Ndc80 in ~80% of cells. Deletion of *LRS4* (Figure 6, A and B) or *CSM1* (Figure 6, C and 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 7, A and 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 to promote the coorientation 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 also contributes to the coorientation process (Figure 7B). Our results also raise the interesting possibility that assembly of the monopolin complex is stepwise, 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 toward one spindle pole. Mam1 could be needed to stabilize these linkages.

Condensins do not appear to be involved in meiosis I sister kinetochore coorientation in other eukaryotes. In *Drosophila melanogaster* hypomorphic alleles in the gene encoding the non-SMC condensin subunit DCAP-G (homologous to *Saccharomyces cerevisiae* Ycg1) do not show defects in coorientation (Resnick *et al.* 2009). *Caenorhabditis 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 coorientation 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 coorientation. In fission yeast, meiotic cohesin complexes associate with the core centromere where they, together with the coorientation factor Moa1, facilitate a kinetochore geometry that favors sister kinetochore coorientation (Yokobayashi and Watanabe 2005; Sakuno *et al.* 2009). In maize and Arabidopsis, cohesins are also essential for sister kinetochore coorientation (Yu and Dawe 2000; Chelysheva *et al.* 2005). Clearly, SMC protein-containing complexes play critical roles in establishing coorientation 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 coorientation reflects differences in kinetochore architecture. Organisms in which sister kinetochore coorientation requires cohesins have regional centromeres with large pericentromeres that are heterochromatic in nature. In contrast, budding yeast centromeres are only 147 bp in length and lack pericentromeric heterochromatin. Structural rigidity appears necessary for sister kinetochore coorientation (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 pericentromeric regions and linking them to centromeric proteins, namely Lrs4 and Csm1, condensins could promote sister kinetochore coorientation.

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 coorientation 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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GENETICS

Supporting Information
http://www.genetics.org/cgi/content/full/genetics.109.115139/DC1

Condensins Promote Coorientation of Sister Chromatids During Meiosis I in Budding Yeast

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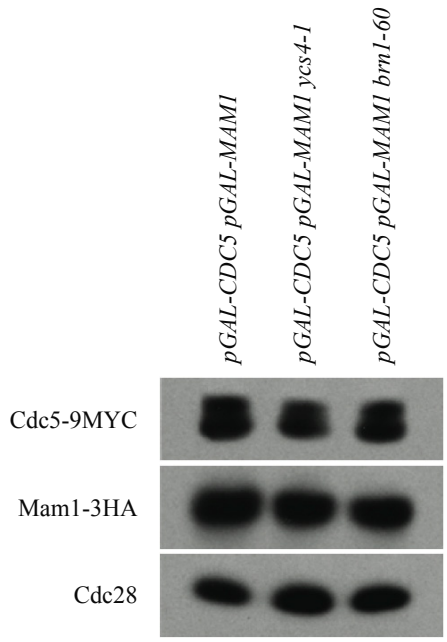

FIGURE S1.— Cdc5 and Mam1 induction from the *GAL1-10* promoter in *ycs4-1* or *brn1-60* cells. Exponentially growing wild-type (A12312), *ycs4-1* (A20739) and *brn1-60* (A21712) cells carrying *pGAL-3HA-MAM1* and *pGAL-3MYC-CDC5* fusions were treated with galactose for 1 hour at 25ºC and then shifted to 34ºC. Samples were taken after 90 minutes to determine the levels of Cdc5 and Mam1 protein. Cdc28 was used as a loading control.

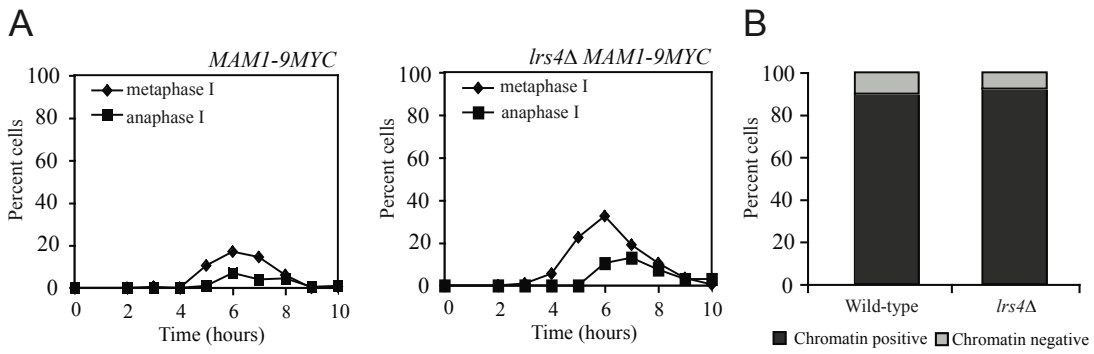

FIGURE S2.— Lrs4 is not required for Mam1 association with chromatin. Wild-type (A7097) and *lrs4*Δ (A24443) cells carrying Mam1-9MYC and Ndc10-6HA fusions were induced to sporulate at 30°C. At the indicated times, samples were taken to determine the percent of cells in metaphase I (diamonds) or anaphase 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 *lrs4* Δ cells (Rabitsch et al, 2003) it associates with chromatin. At least 50 cells were counted per strain.

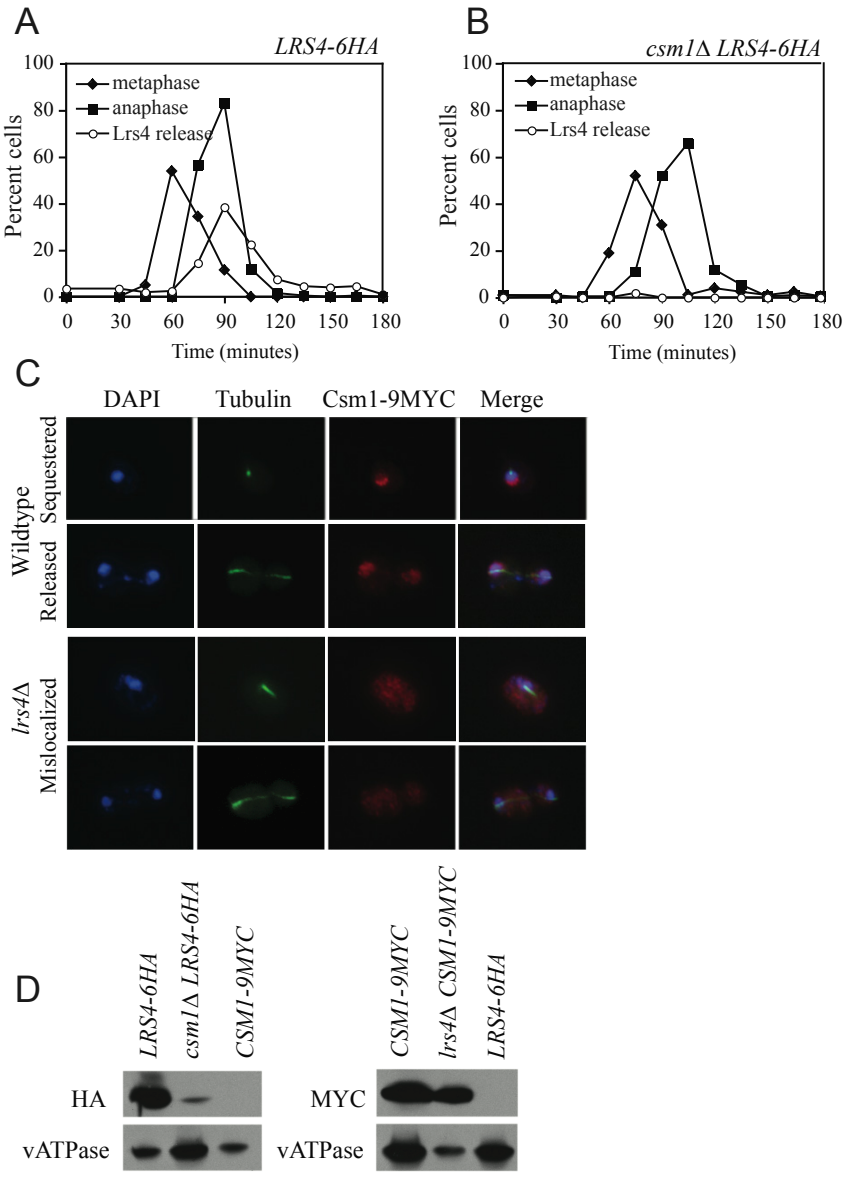

FIGURE S3.— The localization of Lrs4 and Csm1 is interdependent. (A-B) Wild-type (A13838; A) and *csm1*Δ (A15974; B) cells carrying an Lrs4-6HA fusion were released from a pheromone-induced G1 arrest at 25°C. At the indicated times, samples were taken to determine the percentage of cells with metaphase (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 *csm1* Δ 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 *lrs4*Δ 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 *csm1*Δ (A15974) cells at 25°C. Right: Levels of Csm1-9MYC in exponentially growing wild-type (A15087) and *lrs4*Δ (A15976) cells at 25°C. vATPase was used as a loading control.

TABLE S1

Strain derivatives of W303 used in this study

Strain	Relevant genotype
A2587	MATa, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+,*
A5244	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3*
A12312	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, pGAL-3HA-MAM1::KanMX6, pGAL-3MYC-CDC5::URA3*
A13838	MATa, *LRS4-6HA::HIS3MX6*
A15087	MATa, *csm1::CSM1-9MYC::TRP1*
A15910	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, pGAL-3HA-MAM1::KanMX6, pGAL-3MYC-CDC5::URA3 lrs4::HIS3MX6*
A15974	MATa, *LRS4-6HA::HIS3MX6, csm1::KanMX6*
A15976	MATa, *csm1::CSM1-9MYC::TRP1, lrs4::KanMX6*
A20336	MATa, *YCS4-13MYC::KanMX, NDC80-GFP-URA3, cdc14-3*
A20739	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, pGAL-3HA-MAM1::KanMX6, pGAL-3MYC-CDC5::URA3, ycs4-1*
A21128	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, pGAL-3HA-MAM1::KanMX6, pGAL-3MYC-CDC5::URA3, lrs4::HIS3MX6, csm1::KanMX6*
A21607	MATa, *YCS4-13MYC::KanMX, NDC80-GFP-URA3, cdc14-3, lrs4::KanMX6*
A21688	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, brn1-60*
A21712	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, pGAL-3HA-MAM1::KanMX6, pGAL-3MYC-CDC5::URA3, brn1-60*
A21818	MATa, *pURA3-TetR-GFP::LEU2, CenIV::tetOx448::URA3, ycs4-1*
A21860	MATa, *SMC4-13MYC::HIS3MX6, NDC80-GFP-URA3, cdc14-3*
A21861	MATa, *SMC4-13MYC::HIS3MX6, NDC80-GFP-URA3, cdc14-3, csm1::KanMX*

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

TABLE S2

Strain derivatives of SK1 used in this study

Strain	Relevant genotype
A4962	MATa/α, *ho::LYS2/ho::LYS2, ura3/ura3, leu2::hisG/leu2::hisG, trp1::hisG/trp1::hisG*
A7097	MATa/α, *NDC10-6HA::HIS3MX6/NDC10-6HA::HIS3MX6, MAM1-9MYC::TRP1/ MAM1-9MYC::TRP1*
A7118	MATa/α, *pCLB2-CDC20::KanMX6/pCLB2-CDC20::KanMX6, leu2::pURA3-TetR-GFP::LEU2/+, CENV::tetOx224::HIS3/+*
A7316	MATa/α, *pCLB2-CDC20::KanMX6/pCLB2-CDC20::KanMX6, leu2::pURA3-TetR-GFP::LEU2/+, CENV::tetOx224::HIS3/+, mam1::TRP1/mam1::TRP1*
A9043	MATa/α, *NDC10-6HA::HIS3MX6/NDC10-6HA::HIS3MX6, LRS4-13MYC::KanMX6/LRS4-13MYC::KanMX6*
A22517	MATa/α, *NDC10-6HA::HIS3MX6/NDC10-6HA::HIS3MX6, MAM1-9MYC::TRP1/MAM1-9MYC::TRP1, pCLB2-3HA-BRN1::KANMX4/pCLB2-3HA-BRN1::KANMX4*
A22520	MATa/α, *pCLB2-CDC20::KanMX6/pCLB2-CDC20::KanMX6, leu2::pURA3-TetR-GFP::LEU2/+, CENV::tetOx224::HIS3/+, pCLB2-3HA-BRN1::KanMX4/pCLB2-3HA-BRN1::KanMX4*
A23218	MATa/α, *pCLB2-CDC20::KanMX6/pCLB2-CDC20::KanMX6, leu2::pURA3-TetR-GFP::LEU2/+, CENV::tetOx224::HIS3/+, ycg1-2::KanMX4/ycg1-2::KanMX4*
A23220	MATa/α, *pCLB2-CDC20::KanMX6/pCLB2-CDC20::KanMX6, leu2::pURA3-TetR-GFP::LEU2/+, CENV::tetOx224::HIS3/+, ycs4-2/ycs4-2*
A23861	MATa/α, *NDC10-6HA::HIS3MX6/NDC10-6HA::HIS3MX6, LRS4-13MYC::KanMX6/LRS4-13MYC::KanMX6, pCLB2-3HA-BRN1::KANMX4/pCLB2-3HA-BRN1::KANMX4*
A24443	MATa/α, *lrs4D::HIS3/lrs4D::HIS3, NDC10-6HA::HIS3MX6/NDC10-6HA::HIS3MX6, MAM1-9MYC::TRP1/MAM1-9MYC::TRP1*
HY1143	MATa/α, *ura3/ura3, leu2/leu2, his3:KAN/his3:JAN, BRN1-3HA::HIS5/ BRN1-3HA::HIS5*
HY3069C	MATa/α, *ura3/ura3, leu2/leu2, brn1::pCLB2-3HA-BRN1::Kan/ brn1::pCLB2-3HA-BRN1::Kan*

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