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The core centromere and Sgo1 establish a 50-kb cohesin-protected domain around centromeres during meiosis I

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The stepwise loss of cohesins, the complexes that hold sister chromatids together, is required for faithful meiotic chromosome segregation. Cohesins are removed from chromosome arms during meiosis I but are maintained around centromeres until meiosis II. Here we show that Sgo1, a protein required for protecting centromeric cohesins from removal during meiosis I, localizes to cohesin-associated regions (CARs) at the centromere and the 50-kb region surrounding it. Establishment of this Sgo1-binding domain requires the 120-base-pair (bp) core centromere, the kinetochore component Bub1, and the meiosis-specific factor Spo13. Interestingly, cohesins and the kinetochore proteins Iml3 and Chl4 are necessary for Sgo1 to associate with pericentric regions but less so for Sgo1 to associate with the core centromeric regions. Finally, we show that the 50-kb Sgo1-binding domain is the chromosomal region where cohesins are protected from removal during meiosis I. Our results identify the portions of chromosomes where cohesins are protected from removal during meiosis I and show that kinetochore components and cohesins themselves are required to establish this cohesin protective domain.

Keywords: Centromere; cohesion; meiosis; pericentromere; Rec8; Sgo1

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The meiotic cell division cycle is a specialized cell cycle that creates haploid gametes in sexually reproducing organisms. In contrast to the mitotic cell divisions, where DNA replication and chromosome segregation alternate, two consecutive segregation phases follow a single round of DNA replication during the meiotic cell division. Homologous chromosomes segregate from each other during meiosis I, and sister chromatids are partitioned during meiosis II.

Protein complexes known as cohesins play an important role in both mitotic and meiotic chromosome segregation (for review, see Nasmyth 2001). They hold sister chromatids together until the onset of chromosome segregation. In the budding yeast Saccharomyces cerevisiae, the mitotic cohesin complex consists of Smc1, Smc3, Scc1/Mcd1, and Scc3 and associates with chromosomes in a nonuniform manner with peaks of binding enriched at kinetochores, AT-rich sequences, and convergent intergenic regions (Glynn et al. 2004; Lengronne et al. 2004; Weber et al. 2004). The removal of cohesin complexes from chromosomes marks the onset of anaphase and requires the cleavage of the Scc1/Mcd1 subunit by the protease Separase (for review, see Nasmyth 2001).

Meiotic cohesin complexes in yeast are also composed of Smc1, Smc3, and Scc3, but the Scc1/Mcd1 subunit is replaced by the meiosis-specific variant Rec8 (Klein et al. 1999). The distribution of meiotic cohesins along chromosomes is similar to that of mitotic cohesion complexes (Glynn et al. 2004); however, an important difference exists between the ways in which mitotic and meiotic cohesins are removed from chromosomes. Whereas during mitosis cohesins are removed from chromosomes along their entire length at the metaphase–anaphase transition, cohesins are lost from meiotic chromosomes in a step-wise manner. At the metaphase I–anaphase I transition, cohesins are removed from chromosome arms but are maintained around centromeres until anaphase II. Loss of cohesins from chromosome arms is essential for the resolution of chiasmata that hold homologous chromosomes together on the metaphase I spindle (Buonomo et al. 2004). Maintenance of cohesins around centromeres beyond anaphase I and their loss at the metaphase II–anaphase II transition are...
required for the faithful segregation of sister chromatids during meiosis II.

We know of several factors responsible for maintaining cohesins around centromeres beyond meiosis I. The first factor implicated in this process was the Drosophila protein MEI-S332 [Kerrebrock et al. 1992, 1995; Tang et al. 1998]. Subsequent studies in fission and budding yeast, metazoans as well as plants, identified homologs of MEI-S332, termed Sgo1 [Katis et al. 2004a; Kitajima et al. 2004; Marston et al. 2004; Rabitsch et al. 2004; Salic et al. 2004; McGuinness et al. 2005]. In both fission and budding yeast, deletion of SGO1 results in premature loss of Rec8 from centromeric regions during anaphase I and a random meiosis II chromosome segregation pattern. The MEI-S332 proteins themselves localize to centromeric regions [Kerrebrock et al. 1995; Katis et al. 2004a; Kitajima et al. 2004; Marston et al. 2004; Rabitsch et al. 2004; Salic et al. 2004; McGuinness et al. 2005], which requires, in at least fission yeast and humans, the kinetochore and spindle checkpoint component Bub1 [Kitajima et al. 2004, 2005; Tang et al. 2004]. Consistent with this role in regulating Sgo1 localization, cells lacking BUB1 lose centromeric cohesin prematurely [Bernard et al. 2001; Kitajima et al. 2004, 2005]. Another factor required for protecting cohesins from removal around centromeres during meiosis I is the meiosis-specific protein Spo13 [Klein et al. 1999; Lee et al. 2002, Katis et al. 2004b; B.H. Lee et al. 2004]. Spo13 is also necessary for the co-orientation of sister kinetochores, that is, the attaching of sister kinetochores to microtubules emanating from the same spindle pole in meiosis I [Katis et al. 2004b; B.H. Lee et al. 2004].

Immunolocalization studies of cohesins on anaphase I chromosomes show that cohesins are protected from removal during meiosis I at regions overlapping with centromeres [Klein et al. 1999; Watanabe and Nurse 1999; Lee et al. 2003]. Where exactly on chromosomes cohesins are protected, however, is not known in any organism. In eukaryotes in which the core centromere is pericentric heterochromatic regions and appear excluded from the core centromere [Kitajima et al. 2004]. Cytological studies in the fruit fly Drosophila melanogaster and mouse spermatocytes also suggest that cohesins are protected in regions that flank the core centromere [Kerrebrock et al. 1995; Moore et al. 1998; Blower and Karpen 2001; Lee et al. 2003]. Budding yeast centromeres differ greatly from those of other eukaryotes. They are composed of a 120-base-pair (bp) conserved DNA sequence but lack flanking repeat elements. Whether protection of cohesins during meiosis I occurs only at the minimal centromere or whether it is maintained pericentrically in order to ensure functional cohesion between sister chromatids until anaphase II is not known.

Here we show that budding yeast Sgo1 localizes only to cohesin-associated regions (CARs) within a 50-kb region surrounding centromeres previously shown to exhibit enhanced cohesin association during mitosis [Weber et al. 2004]. The association of Sgo1 with the core centromere and pericentric CARs requires the 120-bp core centromere as well as the spindle checkpoint protein Bud1 and the meiosis-specific factor Spo13. Binding of Sgo1 to pericentric CARs depends on cohesins themselves as well as the kinetochore components Iml3 and Chl4. Finally, we show that the CARs within the 50-kb pericentric region where Sgo1 associates are identical to the CARs where cohesins are protected from removal during meiosis I. Our studies define for the first time at the molecular level where on chromosomes cohesins are protected from removal during meiosis I and show that in budding yeast Spo13, the kinetochore components Bud1, Chl4, and Iml3 and cohesin itself function through Sgo1 to establish a chromatin domain where cohesin removal is prevented.

Results

Sgo1 localizes to cohesin-associated sites within a 50-kb region surrounding the centromere during meiosis I

To obtain a molecular understanding of the chromosomal location where cohesins are protected from removal during meiosis I, we first compared the distribution of the cohesin protector Sgo1 with that of the cohesin subunit Rec8 using genome-wide location analysis. Shortly prior to the onset of the first meiotic division (5 h after transfer into sporulation medium) [Fig. 1A; see Materials and Methods], Rec8 was enriched at many regions along the arms of all 16 chromosomes and showed increased association with pericentric regions [Fig. 1C; Supplementary Fig. 1], which matched with previously published genome-wide mapping of cohesins during mitosis [Glynn et al. 2004; Lengronne et al. 2004; Weber et al. 2004].

Using the same genome-wide location analysis, Sgo1 was found enriched around centromeres on all 16 chromosomes [Fig. 1B,C; Supplementary Fig. 2]. Importantly, Sgo1 was not enriched at all sequences around centromeres. Instead, a comparison of the Sgo1 distribution with that of Rec8 revealed that the Sgo1- and Rec8-binding regions overlapped within a roughly 50-kb region surrounding the centromere but that this similarity in distribution was lost farther away from the centromere [Fig. 1C; Supplementary Figs. 3, 4]. Our results indicate that Sgo1 localizes to the same sites as cohesins within a 50-kb domain surrounding the centromere.

Sgo1 associates with pericentric regions in mitotic cells

Although Sgo1 does not prevent cohesin removal during mitosis, the presence of the protein at pericentric regions
until metaphase raised the possibility that Sgo1 affected the distribution of cohesins. However, we found that in cells lacking SGO1 that were arrested in metaphase by depleting cells of Cdc20, Scc1/Mcd1 association was not affected [Supplementary Fig. 5], indicating that in contrast to mammalian cells [McGuinness et al. 2005], Sgo1 does not affect cohesins during the mitotic divisions in budding yeast.

To determine whether differences in Sgo1 binding to chromosomes were responsible for the different behavior of Sgo1 in mitosis and meiosis, we compared the distribution of Sgo1 on chromosomes between mitotic and meiotic cells. Haploid yeast strains were arrested in metaphase using the microtubule depolymerizing drug nocodazole, and the Sgo1 distribution around the centromere of chromosome III was assessed by chromatin immunoprecipitation (ChIP) using primer sets based on our meiotic Sgo1 genome-wide location analysis [Supplementary Fig. 6A]. Sgo1 localized to both centromeres and previously published regions of mitotic cohesin complex enrichment but failed to localize to negative control regions on the arm of chromosome III [Supplementary Fig. 6B,C]. This distribution was qualitatively similar to the Sgo1 distribution observed in meiotic cells [Supplementary Fig. 6D]. In diploid SK1 cells arrested in metaphase I by depleting Cdc20 [Lee and Amon 2003], Sgo1 was found at pericentric regions but not at arm regions. We note that cells are arrested in metaphase in these experiments and thus Sgo1 distribution could be affected. However, the meiotic distribution of Sgo1 in cells depleted for Cdc20 appeared similar to that obtained by location analysis in a synchronous meiosis [Fig. 1C, Supplementary Fig. 2], suggesting that changes in distribution due to arresting cells are minor, if they exist at all. Our results suggest that the distribution of Sgo1 at pericentric regions is qualitatively similar between metaphase I and nocodazole-treated mitotic cells, both conditions in which sister kinetochores are not under tension. Thus, it is likely that mechanisms other than Sgo1 distribution on chromosomes must be responsible for the different behavior of the protein during mitosis and meiosis I.
Sgo1 association with centromeric and pericentric regions depends on Bub1

Next we examined which factors were necessary for Sgo1 to associate with centromeric and pericentric regions. The spindle checkpoint and kinetochore component Bub1 is required for the association of Sgo1 with chromosomes in fission yeast and mammalian cells (Kitajima et al. 2004, 2005; Tang et al. 2004). We found that Bub1 was also required for Sgo1 localization in budding yeast. Sgo1 accumulated in the nucleus in cells lacking BUB1, indicating that the protein was stable [Fig. 2A]. The protein, however, failed to associate with kinetochores as judged by the lack of colocalization of Sgo1 with the kinetochore component Ndc10 on spread nuclei of mitotic and meiotic cells [Fig. 2B–D].

Spo13 is required for full association of Sgo1 with centromeric and pericentric regions

Spo13, which localizes to pericentric regions, is required to protect cohesins from removal during meiosis I (Klein et al. 1999; Katayama et al. 2004b; B.H. Lee et al. 2004). Previous immunolocalization studies suggested that Sgo1 could associate with centromeric regions in the absence of SPO13, although it was noted that the Sgo1 signal was less intense (B.H. Lee et al. 2004). We confirmed that the Sgo1 signal on chromosome spreads was reduced in spo13Δ cells (Fig. 3A,C) and excluded the possibility that this decrease in Sgo1 association with centromeric regions was due to Sgo1 protein levels being lower in spo13Δ cells (Fig. 3B).

The decrease in Sgo1 localization to centromeric regions in spo13Δ cells was not only obvious on chromosome spreads but also when we analyzed Sgo1 binding along chromosomes using genome-wide location analysis. Wild-type cells and cells lacking SPO13 were induced to sporulate, and the distribution of Sgo1 along chromosomes was analyzed as cells entered the first meiotic division, 5 h after transfer of cells to meiosis-inducing conditions [Fig. 4A]. Sgo1 binding at centromeric and pericentric regions was dramatically reduced in cells lacking SPO13 at all 16 chromosomes [Fig. 4B]. The Sgo1-6HA signal was nevertheless above that detected in cells lacking a tagged version of Sgo1 [Fig. 4B], which was particularly obvious when the centromeric regions of all 16 chromosomes were analyzed together [Fig. 4C]. We conclude that Spo13 is required for Sgo1 to associate with centromeric and pericentric regions and that the low-level association of Sgo1 in spo13Δ mutants by genome-wide location analysis indicates that the cytological analysis likely overestimated that amount of Sgo1 associated with regions surrounding the centromere.

Spo13 was not only necessary for the initial loading of Sgo1 onto centromeric regions but also appeared to be required for maintaining Sgo1 at centromeres. Sgo1 association with chromosomes diminished over time as judged by immunolocalization studies and ChIP analyses.
This is reminiscent of the effects of Spo13 on the kinetochore-orientation factor Mam1. For this factor, too, it was found that Spo13 was required for its maintenance at kinetochores (Katis et al. 2004b; B.H. Lee et al. 2004). Our results indicate that Spo13 is essential for a wild-type level association of Sgo1 with both pericentric and centromeric regions. We furthermore note that the reduced localization, but not complete absence, of Sgo1 in spo13/H9004 cells could also explain the observation that not all Rec8 is lost prematurely in spo13/H9004 cells and that a small fraction of Rec8 persists around centromeres into anaphase I (Klein et al. 1999; Katis et al. 2004b; B.H. Lee et al. 2004).

Association of Sgo1 with pericentric sites requires cohesins and the kinetochore proteins Iml3 and Chl4

Which other factors are necessary for Sgo1 association with chromosomes? Owing to the observation that cohesin and Sgo1 physically interact in S. pombe (Kitajima et al. 2004), we considered the possibility that cohesins themselves were required for the association of Sgo1 with the core centromere and pericentric CARs. To test this, we first examined Sgo1 localization on chromosome spreads of meiotic cells lacking the cohesin subunit Rec8 that were also deleted for SPO11, which allows rec8Δ cells to progress beyond prophase I (Klein et al. 1999). Sgo1 localized to centromeric regions as judged by immunolocalization studies on chromosome spreads, although colocalization was poor and the signal was less intense than in a control strain (Fig. 5A). ChIP analysis revealed that Sgo1 association with the core centromere was reduced in rec8Δ spo11Δ cells (Fig. 5C,D). At a pericentric region the Sgo1 signal was even further reduced and only marginally above background (the signal seen at c281) (Fig. 5C,D). Similar results were obtained in a genome-wide location analysis, which was particularly apparent when all 16 chromosomes were analyzed together (Fig. 5E). Sgo1 was present at reduced levels at the core centromere and absent from pericentric regions. Cohesin was also required for Sgo1 to associate with pericentric regions during mitosis. Temperature-sensitive mcd1-1 cells were synchronized in G1 and then released into medium containing nocodazole at the restrictive temperature of 37°C. As in meiotic cells, Sgo1 associated with the core centromere but failed to efficiently associate with a pericentric CAR (c130) in the absence of functional cohesins (Fig. 5F). Our results suggest that Sgo1 associates with the core centromere in part in a cohesin-independent manner, as has been suggested in higher eukaryotes (Kitajima et al. 2004; J.Y. Lee et al. 2004), but cohesins appear critical for association of Sgo1 with pericentric CARs.

Factors required for Sgo1 localization could be proteins identified in a screen of the yeast knockout collection for genes required for maintaining cohesion around centromeres beyond anaphase I (Marston et al. 2004). Two such proteins are the kinetochore components Iml3 and Chl4.
Cells carrying deletions in either gene lose cohesins around centromeres prematurely during meiosis I (Marston et al. 2004). Analysis of the localization of Sgo1 on chromosome spreads revealed that, like in cells lacking REC8, Sgo1 localized to centromeric regions poorly and many Sgo1 foci failed to colocalize with Ndc10 in the mutants (Fig. 6A). ChIP analysis revealed that Sgo1 association with the core centromere was reduced in iml3Δ/H9004 and chl4Δ/H9004 cells (Fig. 6C,D). At pericentric regions, the Sgo1 signal was only marginally above background (the signal seen at site c281) (Fig. 6C,D). Interestingly, iml3Δ cells exhibited a more severe defect than chl4Δ cells, matching with their reduced ability to protect cohesin beyond metaphase I (Marston et al. 2004). Our results indicate that cohesins, Iml3, and Chl4 are important for Sgo1 to associate with pericentric CARs, but the proteins are not essential for, although they contribute to, the association of Sgo1 with the core centromere.

The core centromere is sufficient to target Sgo1 to adjacent CARs

The requirement for the kinetochore components Iml3 and Chl4 in Sgo1 localization to pericentric sites raised the interesting possibility that the 120-bp centromere and proteins that associate with it function as a seed to establish a Sgo1 domain. To test this idea, we integrated a copy of the chromosome VI centromere at the right arm of chromosome III at the TRX3 locus and simultaneously deleted the native centromere of this chromosome. Introduction of CEN6 led to the association of Sgo1 with previously identified CARs (Weber et al. 2004) flanking the neo-centromere (Fig. 7B). For example, Sgo1 association is most highly enriched at the CAR amplified by the R12 primer set but remains low at the negative control region (c281) despite its location within the pericentromere (Fig. 7). Additionally, Sgo1 was no longer found at CARs flanking the region where the native CEN3 was deleted (Fig. 7B,C). These findings not only demonstrate that the core centromere is sufficient to establish an Sgo1-binding domain around itself but also show that the centromere directs Sgo1 specifically to adjacent CARs and not any specific DNA sequences flanking the native centromere.

Rec8 is protected from removal during meiosis I within the 50-kb Sgo1-binding region surrounding the centromere

The finding that Sgo1 localizes to CARs within a 50-kb region surrounding the centromere of chromosome VI 5 h after transfer into sporulation medium. (C) The binding ratios for Sgo1-6HA averaged across all 16 centromeres. The X-axis shows SGD coordinates relative to the centromere for each chromosome, taking into account centromere orientation. (D) Primer sets corresponding to CARs adjacent to the centromere of chromosome III (CEN3, CARC1, c130, CARC2) and a negative control region on the arm of chromosome III (c281). (E,F) Samples were taken for ChIP from wild-type (WT; A12282) and spo13Δ (A11967) cells. (E) Progression through meiosis as the percentage of wild-type metaphase I (closed diamonds), wild-type anaphase I–metaphase II (closed squares), spo13Δ metaphase I (open diamonds), and spo13Δ anaphase I–metaphase II (open squares) spindles. (F) The fold enrichment for sequences relative to a negative control sequence (c281) at the indicated time points as determined by semiquantitative PCR. Note that the strain deleted for SPO13 is delayed 1 h in entering meiosis.
not prove that this is the chromosomal region where Rec8 is protected from removal during meiosis I. To determine where on chromosomes Rec8 persists until the onset of anaphase II, we compared the distribution of Rec8 on chromosomes between cell populations enriched for metaphase I cells, in which cohesins are pres-
ent along the entire length of the chromosomes, and populations enriched for metaphase II cells, in which cohesins are present only around centromeres. This comparison has not been made before because progression through meiosis is too asynchronous to allow for the isolation of cell populations enriched in these particular cell cycle stages. To circumvent this limitation, we constructed strains that arrest in metaphase II by expressing a nondegradable version of the anaphase inhibitor Pds1 (Pds1db\textsuperscript{H9004}) (Cohen-Fix et al. 1996; Shonn et al. 2000) under the control of the meiosis II-specific SPS1 promoter (pSPS1-PDS1db\textsuperscript{H9004}) (see Materials and Methods). This construct drives PDS1db\Delta expression predominantly during meiosis II and acts in a dominant fashion to delay cells in metaphase II as judged by the analysis of Rec8 distribution. Ten hours after transfer into sporulation medium, 50% of the cohesin-containing cells exhibited cohesin localization at centromeric regions, and 25% of cells exhibited a metaphase II spindle morphology (data not shown) [note that this finding also demonstrates that degradation of Pds1 is required for the metaphase II–anaphase II transition]. Thus, in a ChIP analysis that analyzes the distribution of cohesins in the pSPS1-PDS1db\textsuperscript{H9004} strains, we expect an enrichment of cohesins present around centromeres at later time points than at earlier time points when the majority of cells have not yet reached anaphase I.

Analysis of the immunoprecipitated fractions using genome-wide location analysis (Fig. 8A, B) revealed a higher fold enrichment at centromeric and pericentric regions in PDS1db\Delta-expressing cells enriched for metaphase II cells (Fig. 8A–C). Five hours after transfer into sporulation medium when the majority of pSPS1-PDS1db\Delta cells were in a cell cycle stage prior to ana-

Figure 6. IML3 and CHL4 are required for Sgo1 to associate with pericentric CARs. (A) Wild-type (A10461), chl4\textDelta (A10629), and iml3\textDelta (A10628) diploid strains carrying SGO1-9MYC and NDC10-6HA fusions were sporulated to examine Sgo1 localization by chromosome spreads. Sgo1 is shown in green, Ndc10 in red, and DNA in blue. (B–D) Wild-type (A12282), chl4\textDelta (A13970), and iml3\textDelta (A13971) cells carrying the SGO1-6HA fusion were sporulated along with a strain lacking the tagged protein (A4962). (B) The location of primers used for ChIP analysis. c281 is used as a negative control sequence. Primer set R3 amplifies a region ~800 bp to the right of the core centromere. (C) PCR analysis of ChIP samples harvested 4 h (A4962, A12282) or 5 h (A13970, A13971) after transfer into sporulation medium such that cells would be enriched for a population just prior to metaphase. Note that chl4\textDelta and iml3\textDelta cells are delayed 1 h in entering meiosis. (D) The percent immunoprecipitation (%IP) calculated as the percent of immunoprecipitated DNA signal returned in IP fractions. Each experiment was performed at least twice, and the average percent immunoprecipitation is shown.
cells compared with Cde20-depleted cells [Supplementary Fig. 7]. We conclude that the 50-kb domain surrounding the centromere is not only the region where Sgo1 binds but also the region where cohesins are protected from removal during meiosis I.

**Discussion**

Inhibiting cohesin removal around centromeres during meiosis I is essential for accurate chromosome segregation during meiosis. The family of MEI-S332 proteins, of which S. cerevisiae Sgo1 is a member, is required for this process. Here we show that Sgo1 binds to CARs within a 50-kb region around centromeres that coincides with the chromosomal region where cohesins are protected from removal during meiosis I. Establishment of this Sgo1-binding domain requires the 120-bp core centromere, Bub1, and Spo13. Interestingly, whereas Bub1 and Spo13 are required for Sgo1 association with centromeric and pericentric regions, cohesins and the kinetochore proteins Iml3 and Chl4 are necessary for Sgo1 to associate with pericentric regions, but Sgo1 can load to some extent onto the core centromere in their absence, suggesting a multistep mechanism for Sgo1 to associate with chromosomes.

**Defining centromeric cohesion at a molecular level**

Cytological studies in many organisms have shown that sister chromatid cohesion is not removed at regions overlapping with centromeres during meiosis I [for review, see Miyazaki and Orr-Weaver 1994]. Until now, however, we lacked a molecular understanding of the nature of this region in any organism. In eukaryotes other than budding yeast, the mapping of cohesins around centromeres using ChIP was not possible because the regions flanking the centromere are highly repetitive. Budding yeast pericentric regions are not repetitive, making this organism highly suitable for ChIP analysis. However, meiotic cell cycle progression is too asynchronous to obtain cell populations enriched for cells in which cohesins are solely present around centromeres. The generation of cells that express an anaphase inhibitor specifically during meiosis II enabled us to isolate cell populations enriched for cells in which cohesins are protected from removal during meiosis I. Establishment of this Sgo1-binding domain requires the 120-bp core centromere, Bub1, and Spo13. Interestingly, whereas Bub1 and Spo13 are required for Sgo1 association with centromeric and pericentric regions, cohesins and the kinetochore proteins Iml3 and Chl4 are necessary for Sgo1 to associate with pericentric regions, but Sgo1 can load to some extent onto the core centromere in their absence, suggesting a multistep mechanism for Sgo1 to associate with chromosomes.
Figure 8. Cohesins are protected from removal during meiosis I within the 50-kb pericentromere. A strain carrying the pPS1-PDS1dbA allele (A10008) was sporulated in duplicate. Samples were taken after 5 and 10 h for ChIP. (A) The binding ratios of immunoprecipitated Rec8-3HA for the pericentromere and an arm region of chromosome II. Binding ratios for the samples taken at 5 h are shown (black) as well as those taken at 10 h (red). Binding ratios for Sgo1 (dark gray) taken from the experiment in Figure 1 are also shown in addition to binding ratios for a strain lacking the tag (light gray). (B) The ratio of the binding ratio for Rec8-3HA at 10 h [metaphase II-enriched cells] to the binding ratio at 5 h [metaphase I-enriched cells] for all 16 chromosomes with SGD coordinates relative to the centromere of each chromosome. Regions where the ratio of these binding ratios is high indicate portions of the genome that are more enriched for cohesins at 10 h. Raw data [Ratio Meta I/Ratio Meta II, black] are shown along with a smoothed line [Ratio Meta I/Ratio Meta II, red] created by averaging data over a moving 20-point window. A full “metachromosome” is shown [left] in addition to a version including only the 200 kb surrounding the centromere [right]. (C) The binding ratio for Sgo1-6HA for all 16 chromosomes with SGD coordinates relative to the centromere of each chromosome. The data are identical to that represented in Supplementary Figure 3. Raw data [black] are shown along with a smoothed line created by averaging data over a moving 10-point window. A full “metachromosome” is shown [left] in addition to a version including only the 200 kb surrounding the centromere [right].

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in fission yeast, Sgo1 is found in a complex with Rec8 [Kitajima et al. 2004]. From our ChIP analyses it furthermore appears that Sgo1 levels decrease as the distance from the core centromere increases, raising the interesting possibility that Sgo1 “spreads,” perhaps in a cohesin-dependent manner, from the core centromere to pericentric regions.

The finding that the core centromere is capable of directing proteins such as cohesins and Sgo1 to adjacent sites could also provide an explanation for the observation that neo-centromeres are functional not only during mitosis and meiosis II but also during meiosis I. Several human neo-centromeres have been described that lack any obvious pericentric heterochromatin [Aagaard et al. 2000; Saffery et al. 2000; Amor and Choo 2002]. The ability of the core centromere to establish a cohesin-rich and Sgo1-binding domain around itself would provide an explanation as to why these neo-centromeres are functional.

Association of Sgo1 with chromosomes

Our findings identified several proteins important for Sgo1 to associate with chromosomes. Bub1 and Spo13 are required for the association of Sgo1 with the core centromere and pericentric regions. Rec8, Chl4, and Iml3 are necessary for Sgo1 to associate with pericentric sites but less so for Sgo1 binding to centromeric sites.

The requirement for Bub1 in Sgo1 localization appears to be conserved across species [Kitajima et al. 2004, 2005; Tang et al. 2004]. Whether Bub1 binds to centromeric and pericentric sites to localize Sgo1 or whether the protein functions as an Sgo1 “receptor” at the kinetochore and, together with other kinetochore proteins such as Iml3 and Chl4, directs Sgo1 to adjacent CARs is not yet known. Similarly, whether Bub1’s role in localizing Sgo1 is connected to the protein’s function in the spindle assembly checkpoint remains to be determined.

Spo13 is also important for Sgo1 to associate with both centromeric and pericentric sites. However, in contrast to Bub1, the role of Spo13 in regulating Sgo1 and cohesin removal appears more complex. Chromosome-wide location analysis of Spo13 in budding yeast has shown the protein to predominantly localize to pericentric regions during meiosis I [Katis et al. 2004b], putting Spo13 in the right place at the right time to affect Sgo1 localization. Genetic interactions between Spo13 and components of the APC/C-dependent ubiquitylation machinery suggest that Spo13’s function in regulating Sgo1 is to protect the protein from degradation during meiosis I [Katis et al. 2004b]. This idea is consistent with our observation that in spo13Δ cells, Sgo1 association with centromeric and pericentric regions diminishes over time. Spo13, however, not only regulates Sgo1 localization to prevent premature loss of Sgo1 from chromosomes but also functions to regulate chromosome segregation in an Sgo1-independent manner. Overexpression of SPO13 in mitotic cells causes a cell cycle arrest in metaphase [Lee et al. 2002; Shonn et al. 2002]. This arrest is independent of SGO1 [B.H. Lee et al. 2004], indicating that Spo13 not only functions upstream of Sgo1 but also in parallel to regulate the metaphase-anaphase transition.

In cells lacking IML3, CHL4, or REC8, Sgo1 association with chromosomes is partially disrupted, consistent with the observation that random meiosis II segregation of chromosome V is seen in only a fraction of iml3Δ and chl4Δ cells [Marston et al. 2004]. The association of Sgo1 with the core centromere is reduced in cells lacking IML3, CHL4, or REC8. The effects of deleting these genes on Sgo1 at pericentric regions was more dramatic. Sgo1 levels were reduced to almost background levels and background levels in the case of the rec8Δ at pericentric sites. These results suggest that Sgo1 binding to the core centromere is only in part dependent on these factors but is completely dependent at pericentric CARs. It is possible that cohesin, Iml3, and Chl4 decrease Sgo1 loading onto chromosomes overall. We favor the idea that the partial dependence of Sgo1 localization on IML3, CHL4, and REC8 to the core centromere reflects two modes of association of Sgo1 with this genomic region. Sgo1 binds to CARs near the core centromere in a REC8-, IML3-, and CHL4-dependent manner and in addition to the core centromere in a REC8-, IML3-, and CHL4-independent manner. At pericentric sites, Sgo1 binding is solely dependent on cohesins and ILM3 and CHL4. How these factors collaborate to regulate Sgo1 localization is not known. It is possible that Iml3 and Chl4 promote the association of Sgo1 with cohesins at pericentric CARs. It is also possible that Iml3 and Chl4 affect cohesins, thereby preventing Sgo1 from associating efficiently with chromosomes.

In S. pombe and Drosophila, association of Sgo1 with chromosomes is cohesin-independent [Kitajima et al. 2004, J.Y. Lee et al. 2004]. In contrast, in maize Sgo1 association with regions surrounding the centromere requires Rec8 [Hamant et al. 2005]. Our studies of factors regulating Sgo1 localization suggest that in S. cerevisiae, Sgo1 localization to chromosomes is both cohesin-dependent and cohesin-independent, which reflects two steps in the assembly of Sgo1 onto chromosomes. These two steps could reflect different modes of association of Sgo1 with the core centromere and pericentric CARs. Alternatively, it is possible that the core centromere and kinetochore proteins function as a seed for Sgo1 association with chromosomes. From there, the protein spreads to pericentric CARs, which is mediated by the kinetochore proteins Iml3 and Chl4 and cohesins and perhaps involves sliding of cohesins. Consistent with this model is the finding that the 120-bp core centromere is sufficient to direct Sgo1 to a 50-kb domain around itself. It is tempting to speculate that the core centromere functions as an epigenetic organizer of chromosome segregation. It establishes—in an epigenetic manner—a chromosome domain around itself that is essential for the accurate segregation of the chromosome. On a sequence level, the organization of the S. cerevisiae centromere differs dramatically from that of other eukaryotes, in that budding yeast lacks extensive repeated heterochromatic DNA elements [Bernard et al. 2001; Kitajima et al.
2004, 2005; Tang et al. 2004). Our studies suggest that on a functional level, _S. cerevisiae_ centromeres may not be that different from those of other eukaryotes after all. Budding yeast centromeres are also surrounded by a large chromatin domain that binds proteins essential for accurate chromosome segregation.

### Materials and methods

#### Strains and plasmids

The strains used in this study are described in Supplementary Table 1 and were derivatives of SK1 unless otherwise noted. The _pCLB2-CDC20_ fusion is described in Lee and Amon (2003). The _pSPS1-PDS1dbΔ_ construct was generated by cloning the _SPS1_ promoter upstream of _PDS1_ lacking the destruction box [ _PDS1dbΔ_ ] (Cohen-Fix et al. 1996; Shonn et al. 2000). The construct was integrated at the _PDS1_ locus while maintaining an intact copy of wild-type _PDS1_. _bub1Δ::KanMX6_ was created by a one-step PCR-based gene replacement method (Longtine et al. 1998). _sog1Δ::KanMX6, SGO1-9MYC, and SGO1-6HA_ were described in Marston et al. (2004). _NDC10-6HA_ and _rec8a::KanMX4_ were described in Toth et al. (2000). _REC8-3HA, spo13::hisG, and spo11::URA3_ were described in Klein et al. (1999). The strain carrying an ectopic centromere was created by a dual transformation that included the integration of _CEN6-URA3_ at the TRX3 locus on the arm of chromosome III (Weber et al. 2004) and deletion of the endogenous _CEN3_ in a strain carrying the _SGO1-6HA_ fusion.

#### Sporulation conditions

Cells were grown to saturation in YPD [ _YEP + 2% glucose_ ] for 24 h, 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 at pH 7.0_ ] at OD600 = 1.9 at 30°C to induce sporulation.

#### Genome-wide location analysis

Genome-wide location analyses were performed in duplicate as described in Pokholok et al. (2005). The yeast array (Agilent) contains >41,000 probes designed against the entire yeast genome. In total, the probes cover ~12 Mb of the yeast genome (or 85%). Most of the missing regions (represented by flat lines in the graphs) are telomeric or other highly repetitive regions. For genomic regions that are covered, there is a probe approximately every 266 bp. Binding ratios represent the ratio of signal between differentially labeled immunoprecipitated and input fractions and were normalized such that the median binding ratio for each data set equals unity. For the _Sgo1_ location analysis in Figures 1 and 3, input DNA fractions from the wild-type (WT) and _spo13A_ samples were mixed and split prior to labeling to allow for a normalization to compare IP signals. For the _Rec8_ location analysis in Figure 8, input DNA fractions from the 5- and 10-h samples were mixed and split prior to labeling to allow for a normalization to compare IP signals. All data, except the data shown in Figure 8, are shown after smoothing by calculating a moving average across five data points. Complete data sets for all experiments are available upon request.

#### Whole-cell immunofluorescence

Indirect immunofluorescence was carried out as described in Visintin et al. (1998). Rat anti-tubulin antibodies (Oxford Biotechnology) and anti-rat FITC antibodies (Jackson Immunoresearch) were used at a 1:100 dilution. _Sgo1-9Myc_ was detected using a mouse anti-Myc antibody (BabCO) at a 1:2000 dilution and an anti-mouse Cy3 secondary antibody (Jackson Immunoresearch) at a 1:2000 dilution.

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Centromeric cohesion in budding yeast


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**References**


