



Shugoshin protects centromere pairing and promotes segregation of nonexchange partner chromosomes in meiosis

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Faithful chromosome segregation during meiosis I depends upon the formation of connections between homologous chromosomes. Crossovers between homologs connect the partners, allowing them to attach to the meiotic spindle as a unit, such that they migrate away from one another at anaphase I. Homologous partners also become connected by pairing of their centromeres in meiotic prophase. This centromere pairing can promote proper segregation at anaphase I of partners that have failed to become joined by a crossover. Centromere pairing is mediated by synaptonemal complex (SC) proteins that persist at the centromere when the SC disassembles. Here, using mouse spermatocyte and yeast model systems, we tested the role of shugoshin in promoting meiotic centromere pairing by protecting centromeric synaptonemal components from disassembly. The results show that shugoshin protects the centromeric SC in meiotic prophase and, in anaphase, promotes the proper segregation of partner chromosomes that are not linked by a crossover.

shugoshin | meiosis | nonexchange chromosome | centromere pairing | PP2A

Faithful chromosome segregation during meiosis depends upon the formation of connections between homologous chromosome pairs. Crossovers, also called exchanges, are the basis of these connections. Chiasmata, the cytological manifestation of crossovers, and sister chromatid cohesion distal to the crossover create a physical link that holds homologous chromosomes in pairs called bivalents (reviewed in ref. 1). The linkage allows the bivalent to attach to the meiotic spindle as a single unit, such that at anaphase I, the partners will migrate away from one another to opposite poles of the spindle. However, sometimes, even in the absence of exchanges, proper meiotic chromosome segregation is achieved (reviewed in ref. 2). In yeast and *Drosophila* when a single chromosome pair does not experience an exchange, it still usually segregates correctly (3–6). Although nonexchange chromosomes have been difficult to study in mammalian models, there may be mechanisms beyond crossing-over that help to direct their behavior in meiosis I. For example, in mice, the majority of chromosomes in oocytes from Mlh1 recombination-deficient mutants appeared to be spatially balanced on the spindle (7), and in humans, while smaller chromosomes fail to experience crossovers in about 5% of meioses (8–10), they are estimated to nondisjoin in less than 1% of meioses (9–11).

In yeast and *Drosophila*, the centromeres of nonexchange partners pair or interact in meiotic prophase (12–15). Similar centromere pairing is also seen in mouse spermatocytes (16, 17). Meiotic centromere pairing (or clustering in *Drosophila* females) is mediated by proteins that are components of the synaptonemal complex (SC) (14–18). In *Drosophila*, centromere proteins (CENP-C, CAL1) and the meiotic cohesion protein ORD have also been shown to be required for centromere clustering (18–21).

The SC zippers the axes of homologous chromosomes along their lengths in midmeiotic prophase (pachytene) and disassembles in late prophase (diplotene). However, all SC components tested [Zip1 in yeast, its functional homologs SYCP1 in mice, and C(3)G in *Drosophila*; the mouse SC components SYCE1, SYCE2, SYCE3, and TEX12; and the *Drosophila* protein Cona] persist at centromeres, holding them together in pairs (14–18). In budding yeast, this centromere pairing is correlated with proper disjunction of the nonexchange pair (14, 15).

Important questions regarding the mechanism and function of centromere pairing remain unanswered. First, how does centromere pairing in prophase ensure disjunction in anaphase? This is curious, as in budding yeast and mice, the SC components that are protected at the centromeres in late prophase (Zip1/SCYP1) are greatly reduced or undetectable when the centromeres begin attaching to the microtubules of the spindle (14, 16, 17). Second, what enables SC proteins to persist at the paired centromeres when the SC disassembles?

The persistence of centromeric SC in late prophase is reminiscent of the protection of meiotic cohesins at centromeres at the metaphase I to anaphase I transition when arm cohesion is

Significance

Meiotic crossovers form a connection between homologous chromosomes that allows them to attach to the spindle as a single unit in meiosis I. In humans, failures in this process are a leading cause of aneuploidy. A recently described process, called centromere pairing, can also help connect meiotic chromosome partners in meiosis. Homologous chromosomes become tightly joined by a structure called the synaptonemal complex (SC) in meiotic prophase. After the SC disassembles, persisting SC proteins at the centromeres mediate their pairing. Here, studies in mouse spermatocytes and yeast are used to show that the shugoshin protein helps SC components persist at centromeres and helps centromere pairing promote the proper segregation of yeast chromosomes that fail to become tethered by crossovers.

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lost (reviewed in ref. 22). Protection of centromeric cohesin in meiosis I is mediated by shugoshin—a function first revealed by studies of the gene *mei-S322* in *Drosophila* (23, 24). In yeasts and mouse oocytes, shugoshin recruits PP2A phosphatase to centromeres, rendering the centromeric cohesin refractory to cleavage by the protease separase at the metaphase I to anaphase I transition (25–29). Shugoshins also interact with other proteins (Mad2, MCAK, the chromosome passenger complex, and cohesin subunits) to modulate chromosome structure and segregation in meiosis and mitosis (27, 30–34). In budding and fission yeast, shugoshin acts by protecting the Rec8 component of cohesin from phosphorylation by casein kinase and also in budding yeast by the Dbf4 kinase (DDK). In other organisms the identities of the kinases that prepare cohesins for separase cleavage have not been determined (35–37).

Phosphorylation also promotes SC disassembly and degradation, but at the pachytene-to-diplotene transition (reviewed in ref. 38). Studies in rats and mice have correlated the phosphorylation of SC components (SYCP1, SYCP3, TEX12, and SYCE1) with pachytene exit and SC disassembly (39, 40) and the Polo-like kinase PLK1 localizes to the SC central element in pachytene and can phosphorylate SYCP1 and TEX12 in vitro (39). Similarly, in budding yeast, Polo-like kinase (Cdc5) expression is central in promoting SC disassembly (41), but it works in a network with other kinases, namely Dbf-4 kinase and cyclin-dependent kinase (CDK) (39, 42–45).

The parallels between the protection of cohesins and SC components at the centromeres compelled us to explore whether shugoshin is responsible for protecting centromeric SC from disassembly signals upon pachytene exit. Our cytological experiments with mouse spermatocytes revealed that this is the case, while genetic approaches with budding yeast revealed that shugoshin is necessary for mediating the segregation of nonexchange chromosome pairs that depend upon centromere pairing for their meiotic segregation fidelity.

Results and Discussion

Centromere Pairing Links Homolog Pairs in the Absence of Chiasmata.

In chromosome spreads from crossover competent mice about 4–5% of prophase chromosomes appear to be achiasmate (nonexchange) (46–49). Previous studies have shown that homologous partners that appear to be achiasmate are aligned along their arms and are connected at their centromeres by a short block of persisting SC (16, 17). These observations suggest that mechanisms exist to form connections between homologous centromeres without the need for homologous recombination. An alternative explanation is that these apparently nonexchange chromosomes are actually connected by crossovers that do not yield obvious chiasmata in chromosome spreads. If centromere pairing is dependent upon the formation of crossovers that cannot be visualized in chromosome spreads, then in mutants unable to form crossovers, centromere pairing should be greatly reduced. To test this, we examined centromere pairing in mice with mutations that compromise the two major crossover formation pathways. In mice, *Hfm1* is essential to the formation of class I crossovers. This pathway gives rise to ~90% of crossovers (class I crossovers) (50, 51). *Mus81* is essential for the efficient formation of rarer class II crossovers (52). We immunostained spermatocyte chromosome spreads for SYCP3 and SYCP1, components of the lateral and transverse filaments of the SC, in spermatocytes from wild-type, *Hfm1*^{-/-}, and *Hfm1*^{-/-}/*Mus81*^{-/-} mice. SYCP3 immunostaining enabled visualization of chromosome cores and SYCP1 was used to monitor the persistence of SC components at paired centromeres after SC disassembly (centromere pairing) (16, 17). In each chromosome spread we counted the number of chromosome pairs that exhibited no clear chiasmata, whether or not their centromeres were paired (Fig. 1A and B). As expected, elimination of the class I or class I and class II pathways

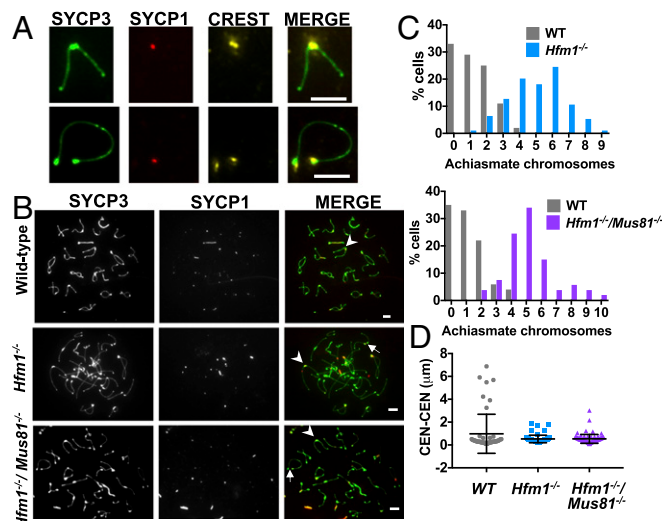


Fig. 1. Centromeres pair efficiently in the absence of chiasmata. (A) Examples of apparently achiasmate partners exhibiting pairing (Top) or no pairing (Bottom) of their centromeres. Indirect immunofluorescence was used to detect SYCP3 (chromosome axes, green), SYCP1 (synaptonemal complex, red), or centromeres (CREST antibody, yellow) in chromosome spreads from mouse spermatocytes. The centromeric end of mouse chromosomes features a bulbous focus of SYCP3 staining and SYCP1 persists at the centromere after SC disassembly (16, 17). (Scale bars, 5 μ m.) (B) Representative mid-diplotene chromosome spreads from wild-type, *Hfm1*^{-/-}, and *Hfm1*^{-/-}/*Mus81*^{-/-} mice. Arrowheads indicate apparently achiasmate chromosomes with paired centromeres. Arrows indicate apparently achiasmate chromosome partners with unpaired centromeres. (C) Achiasmate chromosome frequency in spermatocytes from wild-type and recombination-deficient mice. Chromosome spreads were scored for the number of clearly achiasmate chromosomes per cell (some chromosomes could not be clearly resolved, and thus the graphs underestimate the achiasmate frequency). Chromosome spreads were from wild-type and mutant littermates. (C, Top) WT (gray, $n = 100$ cells) and *Hfm1*^{-/-} mutants (blue, $n = 94$ cells). (C, Bottom) WT (gray, $n = 51$ cells) and *Hfm1*^{-/-}/*Mus81*^{-/-} mutants (purple, $n = 53$ cells). (D) The distance between the centromeres was measured for apparently achiasmate partners in the chromosome spreads used for the experiment in C. WT, $n = 51$ chromosomes; *Hfm1*^{-/-}, $n = 69$ chromosomes; *Hfm1*^{-/-}/*Mus81*^{-/-}, $n = 87$ chromosomes.

resulted in many more achiasmate chromosomes per cell (Fig. 1C) (50, 51). If centromere pairing of apparently achiasmate chromosomes in wild-type mice (16, 17) depends upon undetectable crossovers, then in the recombination mutants achiasmate chromosomes should have a reduced frequency of centromere pairing. To test this, we measured the distance between the centromeres of achiasmate chromosome partners in *WT*, *Hfm1*^{-/-}, and *Hfm1*^{-/-}/*Mus81*^{-/-} mice (Fig. 1D). The loss of recombination did not diminish centromere pairing efficiency (Fig. 1D and *SI Appendix*, Fig. S1). This result suggests that paired centromeres of achiasmate partners are not held together by undetectable crossovers, but rather by persisting SC at the centromeres (16, 17).

Shugoshin 2 Protects Centromere Pairing in Mice. Shugoshin 2 (SGO2) localizes to the centromeres of chromosomes in mouse spermatocytes (53). We compared the centromeric localization of SGO2 with the timing of SC protection at centromeres (Fig. 2A). The axial/lateral element component SYCP3 was used as a marker for the SC. SGO2 is first detected at centromeres in early diplotene cells (Fig. 2A and B) and is still there in mid- and late diplotene. This corresponds with the time at which SC components are removed from chromosome arms but not from centromeres (16, 17). Thus, these data are consistent with the model that SGO2 protects the centromeric SC from the disassembly process.

To test whether SGO2 is necessary for the protection of centromeric SC, we monitored the persistence of centromeric

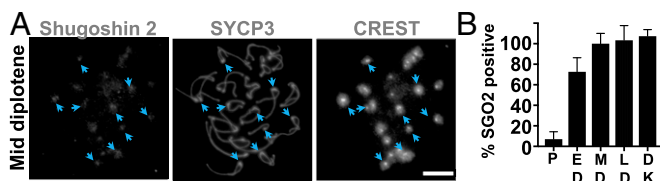


Fig. 2. SGO2 colocalizes with persisting synaptonemal complex components at centromeres in prophase I. (A) Indirect immunofluorescence was used to evaluate localization of SGO2 on chromosomes at stages of diplotene. Staining with CREST antibody was used to identify centromere regions. Arrowheads indicate examples of paired centromeres. (Scale bar, 5 μ m for all images.) (B) The average percentage of centromeres per spread showing colocalization of SGO2. DK, diakinesis; ED, early diplotene; LD, late diplotene; MD, mid diplotene; P, pachytene. The stage of each cell was determined by the SC morphology (*Materials and Methods*). Error bars indicate SD. A minimum of 20 spreads were scored for each category.

SYCP1 in *Sgo2*^{-/-} spermatocytes and wild-type control cells (Fig. 3). In wild-type cells SYCP1 persistence at centromeres mirrors that of other SC components (16, 17). In pachytene cells the SC of the *Sgo2*^{-/-} mutants was indistinguishable from the wild-type control (Fig. 3A and B). In early diplotene, the SYCP1 signal was visible at nearly all paired centromeres in wild-type chromosome spreads and at nearly 75% of the centromeres in the *Sgo2*^{-/-} mutants (Fig. 3C). Other SC components, SYCE1, SYCE2, and SIX6OS1, behaved similarly to SYCP1 (*SI Appendix, Fig. S2*). In wild-type spreads the SYCP1 persisted at the centromeres through diplotene (Fig. 3C). But in *Sgo2*^{-/-} mutants the percentage of centromeres with detectable SYCP1 staining was reduced in late diplotene (Fig. 3A–C). Thus, the absence of SGO2 did not detectably affect SC components in pachytene but did allow SC components to be lost from centromeres more quickly than in wild-type spermatocytes.

The heightened loss of SYCP1 from centromeres would predict that *Sgo2*^{-/-} spermatocytes would also have a defect in homologous centromere pairing in diplotene. In early diplotene, nearly all centromeres are paired in wild-type spermatocytes and pairing levels go down as cells proceed through diplotene (Fig. 3A and D). In contrast, by early diplotene in *Sgo2*^{-/-} cells many of the centromere pairs have already disengaged and pair levels go down through diplotene (Fig. 3B and D). In both the wild-type control and the *Sgo2*^{-/-} chromosome spreads, the unpaired centromeres have significantly less SYCP1 staining than do the paired centromeres (Fig. 3E and *SI Appendix, Fig. S3*), supporting the notion that it is the protection of SYCP1 that allows centromere pairing to persist.

Together these results suggest that wild-type and *Sgo2*^{-/-} spermatocytes have similar SC structures and complete centromere pairing in pachytene. Importantly, in early diplotene in *Sgo2*^{-/-} mutants, the SC is present at most centromeres and they remain paired. Thus, SGO2 is not necessary for establishing centromere pairing. However, centromere pairing disappears more rapidly in *Sgo2*^{-/-} spermatocytes, suggesting that SGO2 is necessary to maintain centromeric SC components, and centromere pairing, in diplotene.

PP2A Promotes Centromere Pairing in Mouse Spermatocytes. SGO2 could be protecting centromeric SC through the recruitment of one of its effector proteins to the centromere (reviewed in ref. 54). Shugoshin recruits PP2A phosphatase to meiotic centromeres in germ cells, where the PP2A opposes the phosphorylation of centromeric cohesins (25, 26, 55). To test whether this mechanism is being used to protect centromeric SC from disassembly in diplotene, we evaluated the persistence of centromeric SC, and centromere pairing, in spermatocytes when phosphatase activity was inhibited (Fig. 4). In these experiments we evaluated

diplotene-like chromosome spreads from cultured spermatocytes (56) treated with the phosphatase inhibitors cantharidin and okadaic acid, which at the concentrations used preferentially inhibit PP2A over other phosphatases (57, 58). Treatment with either inhibitor significantly reduced the retention of SYCP1 at the centromeres and resulted in a substantial loss of centromere pairing (Fig. 4A–D), consistent with the model that PP2A protects SYCP1 at centromeres, although it is formally possible that other targets of these compounds could be involved.

Recent studies in *Drosophila* have suggested that PP2A and shugoshin might each act to promote localization of the other to the centromeres (59), but in our experiments no reduction in SGO2 localization was seen at the centromeres following addition of the phosphatase inhibitors (Fig. 4A and D). Although it is possible that both inhibitors are achieving their effects through some other target, the fact that both of these PP2A inhibitors reduce SC protection at centromeres is consistent with the model that PP2A, recruited by SGO2, opposes kinase activities that promote SC disassembly at centromeres upon pachytene exit.

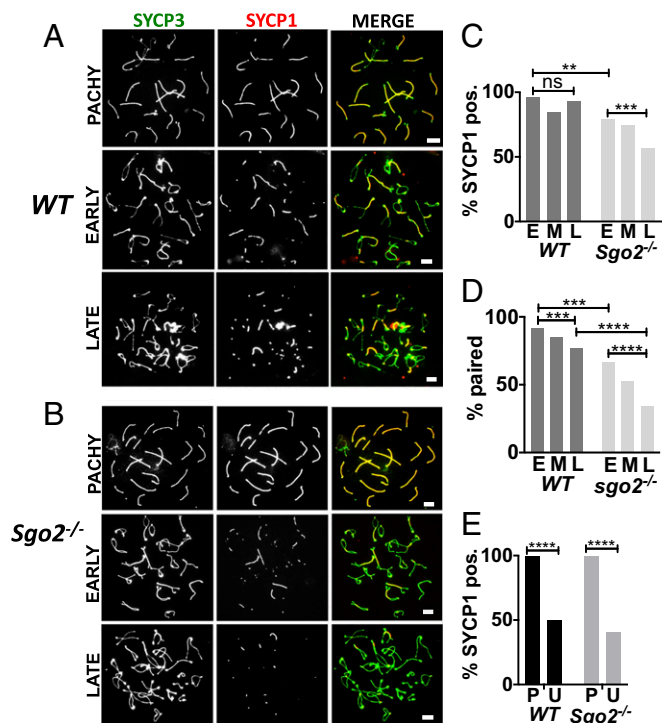


Fig. 3. SGO2 is required for the persistence of centromeric synaptonemal complex components in diplotene. Indirect immunofluorescence was used to monitor the morphology of chromosomes from wild-type and *Sgo2*^{-/-} spermatocytes. (A and B) Representative chromosome spreads from pachytene, early diplotene, and late diplotene (A) wild-type spermatocytes and (B) *Sgo2*^{-/-} spermatocytes. (Scale bars, 5 μ m.) (C) Histogram of SYCP1 localization at centromeres in spreads from wild-type and *Sgo2*^{-/-} spermatocytes in early (E), middle (M), and late (L) diplotene. The numbers of SYCP1-positive centromeres scored were WT early, 50/52; WT middle, 46/54; WT late, 88/95; *Sgo2*^{-/-}, 72/91; *Sgo2*^{-/-}, 114/151; and *Sgo2*^{-/-}, 100/174. (D) Histogram of the percentage of paired centromeres on chromosomes from wild-type and *Sgo2*^{-/-} spermatocytes. The numbers of paired centromeres were WT early, 48/52; WT middle, 46/54; WT late, 67/98; *Sgo2*^{-/-}, 61/91; *Sgo2*^{-/-}, 80/151; and *Sgo2*^{-/-}, 59/174. (E) SYCP1 localization to paired and unpaired centromeres. (E) Paired (P) and unpaired (U) centromeres from all stages of diplotene (D above) were classified according to their SYCP1 staining. The numbers of centromeres scored were WT paired, 167/167; WT unpaired, 17/34; *Sgo2*^{-/-} paired, 200/200; and *Sgo2*^{-/-} unpaired, 89/216. The significance of differences between samples was evaluated using Fisher's exact test. ****P* < 0.01, *****P* < 0.001, ******P* < 0.0001.

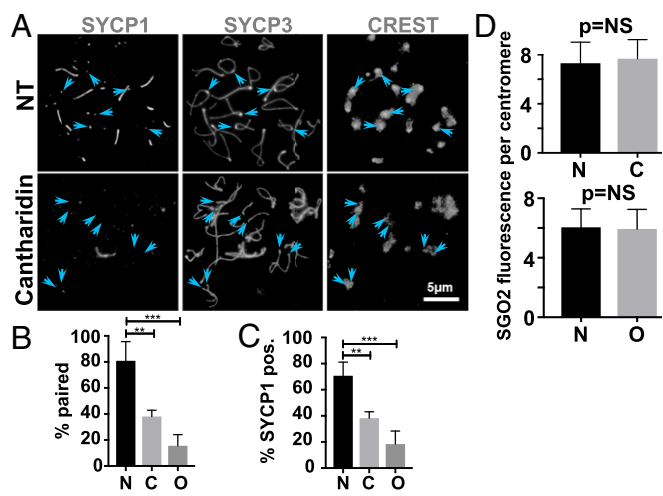


Fig. 4. Phosphatase activity is needed for SYCP1 to persist at centromeres in diplotene. Cultured spermatocytes were treated with okadaic acid or cantharidin. Chromosome spreads were then evaluated using indirect immunofluorescence microscopy. The presence of SYCP1 at centromeres and the fraction of chromosomes in each spread with paired centromeres (stained with CREST antibodies) were scored. (A) Representative images of chromosome spreads that were not treated (NT) or treated with cantharidin. (Scale bar, 5 μ m for all panels.) Arrowheads indicate examples of paired centromeres (Top) and unpaired centromeres (Bottom). One hundred diplotene chromosome spreads were scored for SYCP1 localization to centromeres and centromere pairing. (B) The percentage of chromosomes in each spread with SYCP1 at the centromeres. Averages and SDs are N (not treated), $80.9 \pm 14.7\%$; C (cantharidin), $38.0 \pm 4.9\%$; and O (okadaic acid), $15.5 \pm 8.6\%$. (C) The percentage of chromosomes in each spread with paired centromeres. Averages and SDs are N (not treated), $70.7 \pm 10.4\%$; C (cantharidin), $38.2 \pm 4.9\%$; and O (okadaic acid), $18.4 \pm 10.0\%$. One hundred chromosome spreads were scored for each treatment. Significance was evaluated using Student's *t* test. (D) Histogram showing the relative amount of SGO2 on centromeres of untreated (N) or cantharidin (C)- or okadaic acid (O)-treated spermatocytes. $***P < 0.01$, $****P < 0.001$.

Shugoshin Promotes Disjunction of Nonexchange Chromosomes. In budding yeast, it has been possible to demonstrate directly that centromere pairing in prophase is necessary for subsequent disjunction of nonexchange partner chromosomes in anaphase I (60). Since shugoshin is acting at the centromeres in this interval, we tested whether it is important for the meiotic segregation of nonexchange chromosomes. In the mouse model, there is no established system for following the fate of nonexchange chromosomes, so we addressed this question using budding yeast, which has a single shugoshin gene, *SGO1*. Yeast *sgo1 Δ* (deletion) mutants show only low levels of meiosis I nondisjunction (of exchange chromosomes), but severe defects in meiosis II (61). As was first shown in *Drosophila* (23, 24), the meiosis II defect is due to a failure to protect centromeric cohesion at the metaphase I to anaphase I transition. We first monitored the requirement for *SGO1* for centromere pairing using a pair of centromere-containing plasmids that act as nonexchange minichromosome partners in meiosis (62). These minichromosomes do not experience exchanges, yet they disjoin properly in most meioses (4, 63, 64). Cells bearing the minichromosomes were induced to enter meiosis, chromosome spreads were prepared, and pachytene spreads (as judged by Zip1 morphology) were scored for the association of the two minichromosomes, which were tagged at their centromeres with GFP and tdtomato (Fig. 5A). The distances between the red and green foci marking the minichromosomes was measured in wild-type (*SGO1*) cells and cells that do not express *SGO1* in meiosis (*sgo1-md*) (65). Foci with center-to-center distances of less than 0.6 μ m were as scored as “paired” (as in Fig. 5A, Top) while those farther apart were scored as “unpaired” (as in Fig. 5A, Bottom).

The *sgo1-md* mutants show considerable centromere pairing in pachytene, although at slightly lower levels than the control (Fig. 5B). Deletion of the SC gene *ZIP1* reduces pairing to a few percent in these assays (62). Thus, as was seen in mice (Fig. 3), shugoshin is not essential for establishing centromere pairing. To test whether Sgo1 is necessary for the persistence of centromere pairing after pachytene exit, as it is in spermatocytes (Fig. 3D), cells were synchronously released from a pachytene arrest and centromere pairing was scored. The arrest/release was achieved by using strains in which the *NDT80* meiotic transcription factor was under the control of an estradiol-inducible promoter (43, 66). Following pachytene release cells synchronously pass through diplotene and by 2 h begin entering early metaphase (SI Appendix, Fig. S4). Centromere pairing of minichromosomes tagged with mTurquoise or mVenus was scored (Fig. 5C and D). In pachytene cells (just before addition of estradiol) centromeres were paired in most cells in both wild-type and *sgo1* mutants (Fig. 5D). As in the spermatocytes, centromere pairing levels diminished as cells continued meiotic progression (Fig. 5D). By early metaphase the *sgo1* mutants had significantly lower levels of pairing than the wild-type cells (Fig. 5D). Therefore, as in mouse spermatocytes, centromere pairing is naturally lost following pachytene and shugoshin slows the loss of pairing after pachytene exit.

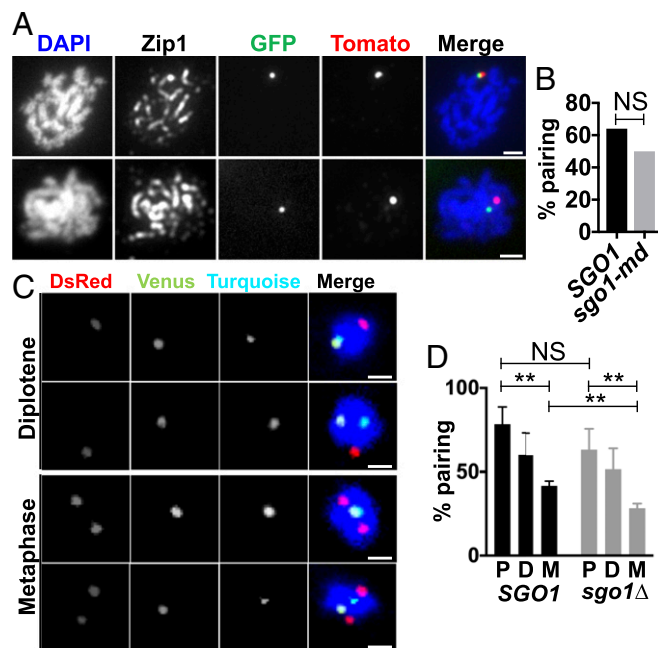


Fig. 5. Shugoshin is not needed for centromere pairing in pachytene but is required for nonexchange segregation. (A) Representative chromosome spreads showing examples of paired (Top) and unpaired (Bottom) minichromosome centromeres (both images from the *sgo1-md* strain). Chromosome spreads were stained with DAPI to show chromatin, anti-Zip1 antibody to show the SC, and anti-GFP and DsRed to show the locations of the centromere-proximal tags. (Scale bars, 1 μ m.) (B) Histogram showing percentage of centromere pairing in each strain (*SGO1*, $n = 50$ spreads; *sgo1-md*, $n = 100$ spreads). (C) Representative micrographs showing examples of paired (Top) and unpaired (Bottom) minichromosome centromeres for both diplotene and metaphase cells (all images from the *SGO1* strain DJE90). Cells expressed mVenus-lacI and tetR-mTurquoise which bound to lac operator and tet operator arrays that were adjacent to the centromeres on the two minichromosomes. Spindle pole bodies are shown in red (*SPC42-DSRed*). Chromosomes were stained with DAPI. (Scale bars, 1 μ m.) (D) Histogram showing percentage of pairing in pachytene, diplotene, and early-metaphase cells (P, D, and M, respectively) in *SGO1* and *sgo1 Δ* cells. Experiments were performed in three replicates of 20 cells each. Statistical comparisons were performed with an unpaired *t* test. For all histograms, NS, not significant and $***P < 0.01$.

In both spermatocytes and budding yeast, centromere pairing occurs in prophase and in yeast it is necessary for nonexchange disjunction in anaphase (14, 15)—even though the pairing has largely dissolved well before anaphase (Fig. 5D). We have shown the centromere pairing is largely intact in shugoshin mutants but shugoshin affects centromere biology after pachytene exit, as centromere pairing and SC structures at centromeres are lost faster in shugoshin mutants.

To determine whether shugoshin is involved in later events in nonexchange centromere behavior, we examined the segregation of nonexchange minichromosomes in anaphase I (Fig. 6A). Fluorescence microscopy was used to determine whether minichromosomes (marked by tdTomato and GFP foci) segregated to opposite poles in anaphase I cells. In the wild-type control, the minichromosomes nondisjoined in about 26% of meioses while in the *sgo1-md* mutants they nondisjoined in ~50% of meioses—consistent with random segregation (Fig. 6B). Thus, although most minichromosomes pair at their centromeres in pachytene in *sgo1* mutants (Fig. 5D), the pairing does not ensure disjunction. We also tested the role of Sgo1 in promoting the disjunction of authentic yeast chromosomes. In these experiments the yeast carried either a normal chromosome *V* pair or a pair of homeologous chromosome *V*s (one from *Saccharomyces cerevisiae* and one from *Saccharomyces bayanus*) that do not experience crossovers in meiosis because of sequence divergence (67). Both chromosome pairs were tagged with GFP at the centromeres.

Deletion of *SGO1* (*sgo1Δ*) resulted in a small increase in non-disjunction frequency of the homologous chromosome *V*s (Fig. 6C), consistent with earlier studies (61). In contrast, the non-exchange, homeologous pair exhibited a significant increase in nondisjunction when *SGO1* was deleted, consistent with nearly random segregation (Fig. 6D).

The PP2A inhibitor experiments suggested that Sgo2 (in mice) acts to protect centromere pairing through recruitment of PP2A. Is Sgo1 of yeast promoting nonexchange disjunction by recruiting PP2A? To test this, we took advantage of the *sgo1-3A* allele in which three critical contact amino acids required for Sgo1 to recruit PP2A to centromeres are converted to alanines (29). This mutant, which exhibits normal loading of Sgo1 to meiotic kinetochores (29), showed random segregation of the nonexchange partners, providing strong support for the model that Sgo1 promotes nonexchange segregation through the recruitment of PP2A to centromeres.

These experiments show that shugoshin is not needed for the establishment of centromere pairing, that centromere pairing dissolves after pachytene exit, and that shugoshin slows the dissolution of centromere pairing. In both nematodes and rice, SCs disassemble prematurely in the absence of shugoshin (30, 34). Thus, it could be that the protection of SC components at centromeres, observed in our studies, reflects a more general conserved role for shugoshins in modulating SC stability and the timing of its disassembly.

While previous work demonstrated that prophase centromere pairing is essential for nonexchange disjunction in yeast (14, 15), the results presented here reveal that centromere pairing is not sufficient to ensure disjunction—since in yeast *SGO1* mutants most centromeres are paired upon pachytene exit. Earlier studies found that in wild-type cells, centromeric SC proteins disappear before chromosomes begin to orient on the spindle in early metaphase (14–17). This observation, coupled with our findings, suggests that centromere pairing might be a precondition for setting up the mechanism that later promotes biorientation in anaphase. Centromere pairing cannot be directly mediating biorientation since SC proteins are normally gone from the centromeres, and centromere pairing is dissolved, before biorientation is accomplished. The observation that nonexchange partners in *Drosophila* appear to be tethered by threads of pericentromeric heterochromatin during the metaphase biorientation process (68) suggests the model that prophase centromere pairing could provide a platform for the establishment of centromeric connections between nonexchange partners. The fact that nonexchange segregation is randomized in yeast shugoshin mutants raises the possibility that shugoshin is not essential for centromere pairing, per se, but instead for the formation or maintenance of a structure or process that promotes biorientation. By this model, the low levels of meiosis I nondisjunction of native chromosomes in shugoshin mutants may reflect the times at which these chromosomes fail to experience exchanges and rely upon a centromere pairing-based mechanism to ensure their disjunction in meiosis I.

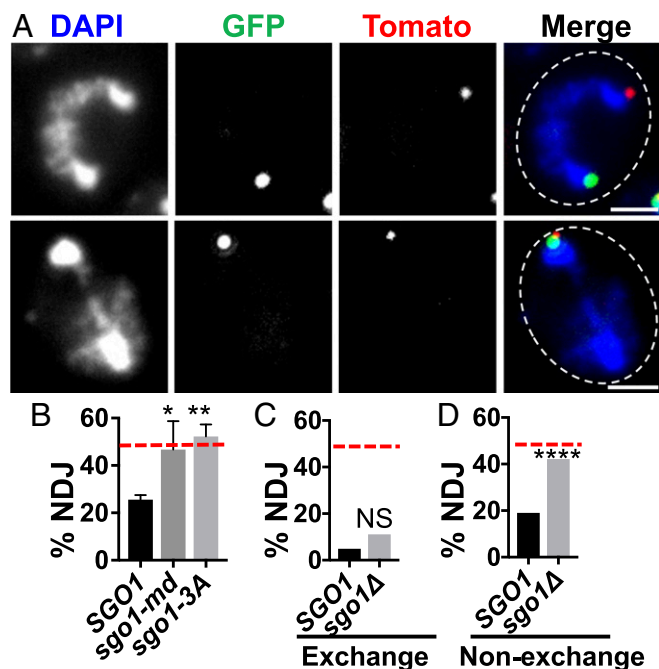


Fig. 6. Shugoshin is required for nonexchange segregation of minichromosomes. (A) Representative anaphase cells showing disjoined (Top) and nondisjoined (Bottom) minichromosomes. Cells were stained with DAPI to show chromatin. Locations of minichromosome centromeres were detected by GFP and tdTomato fluorescence. (Scale bars, 1 μ m.) (B) Histogram showing nondisjunction frequencies of minichromosomes in *SGO1*, *sgo1-md*, and *sgo1-3A* cells (three replicates of 30 cells each were analyzed for all three strains). Statistical comparisons were performed using an unpaired *t* test. (C) Histogram showing nondisjunction frequencies of homologous chromosomes in *SGO1* and *sgo1Δ* cells. ($n = 122$ cells for *SGO1* and 90 cells for *sgo1Δ*.) (D) Histogram showing nondisjunction frequencies of homeologous chromosomes in *SGO1* and *sgo1Δ* cells. ($n = 121$ cells for both strains; nondisjunction frequencies were 19.0% vs. 42.1%, $P < 0.0001$.) (B–D) Red dashed line equals the level of nondisjunction expected for random segregation. (C and D) Statistical comparisons were performed with Fisher’s exact test. For all histograms, NS, not significant and $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$.

Materials and Methods

The mouse and yeast strains, reagents, and methods for this project are described in *SI Appendix*. The Oklahoma Medical Research Foundation Animal Care and Use Committee approved all animal protocols. For mouse cytology experiments, staging of chromosome spreads in diplotene was based on the extent of SYCP1 staining. Established approaches were employed for visualizing chromosomes in surface spreads (16, 50, 69, 70).

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