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Chiasmata Promote Monopolar Attachment of Sister Chromatids and Their Co-Segregation toward the Proper Pole during Meiosis I

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Abstract

The chiasma is a structure that forms between a pair of homologous chromosomes by crossover recombination and physically links the homologous chromosomes during meiosis. Chiasmata are essential for the attachment of the homologous chromosomes to opposite spindle poles (bipolar attachment) and their subsequent segregation to the opposite poles during meiosis I. However, the overall function of chiasmata during meiosis is not fully understood. Here, we show that chiasmata also play a crucial role in the attachment of sister chromatids to the same spindle pole and in their co-segregation during meiosis I in fission yeast. Analysis of cells lacking chiasmata and the cohesin protector Sgo1 showed that loss of chiasmata causes frequent bipolar attachment of sister chromatids during anaphase. Furthermore, high time-resolution analysis of centromere dynamics in various types of chiasmate and achiasmate cells, including those lacking the DNA replication checkpoint factor Mrc1 or the meiotic centromere protein Moa1, showed the following three outcomes: (i) during the pre-anaphase stage, the bipolar attachment of sister chromatids occurs irrespective of chiasma formation; (ii) the chiasma contributes to the elimination of the pre-anaphase bipolar attachment; and (iii) when the bipolar attachment remains during anaphase, the chiasmata generate a bias toward the proper pole during poleward chromosome pulling that results in appropriate chromosome segregation. Based on these results, we propose that chiasmata play a pivotal role in the selection of proper attachments and provide a backup mechanism that promotes correct chromosome segregation when improper attachments remain during anaphase I.

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Introduction

During cell division, chromosomes that harbor genetic information are accurately segregated into daughter cells. Chromosome segregation depends on attachment of chromosomes to the spindle via chromosomal sites called kinetochores. The interaction between kinetochores and spindle microtubules, which extend from opposite spindle poles, generates pulling forces on the chromosomes from opposite directions, causing them to migrate toward opposite spindle poles. To understand the mechanisms underlying chromosome segregation, it is crucial to elucidate how chromosomes attach to the spindle.

In mitosis, sister chromatids are segregated to opposite poles (equational segregation; Figure 1). The sister chromatids are associated until anaphase via a protein complex called cohesin [1,2], which is required for the back-to-back arrangement of the kinetochores that permits their attachment to opposite spindle poles [3]. In addition, when sister chromatids are pulled from opposite directions, the cohesion generates tension at the kinetochore that leads to stabilization of the kinetochore–microtubule interaction, probably via inactivation of aurora kinase [4]. When the cohesion is compromised, sister chromatids fail to attach to the spindle properly and are mis-segregated [5–8].

During meiosis, on the other hand, a physical association between homologous chromosomes additionally contributes to proper spindle attachment of chromosomes [3,9,10]. Meiosis occurs during gamete formation, and during meiosis, two rounds of chromosome segregation follow a single round of DNA replication, resulting in the production of gametes with half the original number of chromosomes. Chromosome segregation during meiosis I is specific to meiosis: Homologous chromosomes attach to opposite spindle poles, with each pair of sister chromatids attaching to the same pole (monopolar attachment), and are segregated to the opposite poles (reductional segregation; Figure 1). As in mitosis, sister chromatid cohesion is required for proper kinetochore arrangement during meiosis. However, a meiosis-specific type of cohesin mediates this cohesion [11–15], and sister kinetochores are arranged side by side facing the same direction so that they become attached to the same pole [16]. Furthermore, shugoshin proteins maintain centromeric cohesion during ana-

Author Summary

Gametes form through a special type of cell division called meiosis. During meiosis, two nuclear divisions take place successively; the first division is specific only to meiosis, in that homologous chromosomes segregate from each other. Homologous chromosome segregation requires physical association of the homologous chromosomes by a structure called chiasma that forms at the site of recombination. This association is thought to contribute to proper attachment of homologous chromosomes to the spindle, leading to their proper segregation. In this study, we examined the functions of chiasmata during the first division in fission yeast by analyzing chromosome dynamics and segregation in several different mutants lacking chiasmata. We found that, in addition to proper spindle attachment of homologous chromosomes, chiasmata contribute to proper spindle attachment of replicated chromosomes more substantially than previously had been thought. In addition, even when chromosomes are improperly attached to the spindle, chiasmata eventually cause proper chromosome segregation. Our findings reinforce the significance of the physical association of homologous chromosomes in proper spindle attachment of chromosomes and have unveiled a previously unidentified, chiasma-dependent mechanism that ensures proper chromosome segregation.

phase I [17–21]. These proteins inhibit the removal of centromeric cohesin and regulate centromeric aurora kinase [17–19,21–24]. Elimination of both of these functions compromises sister chromatid segregation during meiosis I and II [17,18,22,25]. Further, elimination of the cohesin-retention function alone causes sister chromatid separation after anaphase I but has little if any effect on sister chromatid segregation toward the same pole during anaphase I [17,19]. Unlike the situation in mitosis, homologous chromosome association contributes to the generation of tension at the kinetochore in meiosis. Homologous chromosomes are physically associated with each other via the chiasmata that are formed by reciprocal recombination. When homologous chromosomes are pulled in opposite directions, the chiasmata generate tension at the kinetochore and stabilize the kinetochore–microtubule interaction. Elimination of chiasmata leads to non-disjunction of homologous chromosomes [26].

In addition to this widely accepted role, chiasmata appear to play additional roles in the attachment of chromosomes to the spindle. A lack of chiasmata results in the separation or fragmentation of sister chromatids during meiosis I in many species [27–29], suggesting that chiasmata prevent the bipolar attachment of sister chromatids. Furthermore, chiasmata greatly alter meiotic sister chromatid segregation patterns in several different types of fission yeast cells. Fission yeast cells normally undergo meiosis after responding to the mating pheromone [30], but meiosis can also be induced without mating pheromone response by inactivation of Pat1 kinase, a key negative regulator of meiosis [31,32]. We previously reported that when haploid fission yeast cells lacking homologous chromosomes were forced to enter meiosis by Pat1 inactivation after a mating pheromone response, sister chromatids were primarily segregated to the same pole at meiosis I, as seen in normal diploid meiosis [33]. However, when they were induced to enter meiosis without a mating pheromone response, sister chromatids primarily underwent equational segregation. By contrast, when Pat1 inactivation forced diploid cells to enter meiosis without a mating pheromone response, the sister chromatids were primarily segregated to the same pole in a recombination-dependent manner. Similar recombination-dependent co-segregation of sister chromatids has been observed in several cohesin-related mutants of fission yeast [34]. These findings suggest that chiasmata promote the monopolar attachment of sister chromatids; however, because a loss of recombination causes only a negligible level of equational segregation during normal diploid meiosis in fission yeast cells, chiasmata have previously been thought to be dispensable for monopolar

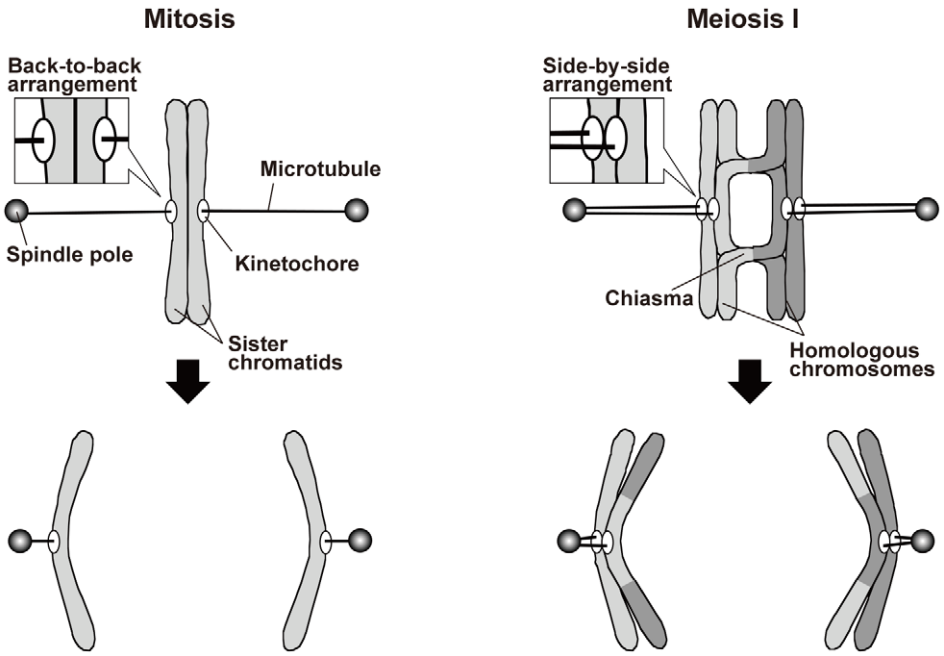

Figure 1. Spindle attachment of chromosomes and their segregation during mitosis and meiosis I. For simplicity, only a single kinetochore-interacting microtubule is shown for each kinetochore, and other microtubules are not shown.
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attachment of sister chromatids [35]. The contribution of chiasmata to the monopolar attachment during meiosis I, therefore, remains elusive.

To understand the mechanisms underlying meiotic chromosome segregation, we examined the functions of chiasmata in spindle attachment and segregation of sister chromatids during meiosis I in fission yeast. Our analysis of chromosome segregation and dynamics in several different types of achiasmate cells showed that in the absence of chiasmata, sister chromatids were frequently attached to opposite poles during anaphase I. High time-resolution analysis of centromere dynamics further showed that chiasmata contribute to the elimination of bipolar attachments during the pre-anaphase stage. Furthermore, when the bipolar attachments remain during anaphase I, chiasmata induce a bias toward the proper pole during poleward chromosome pulling from opposite directions that results in correct chromosome segregation. Based on our findings, we discuss how chiasmata contribute to spindle attachment and segregation of chromosomes and further extend our idea to include the general functions of chromosome association during mitotic and meiotic chromosome segregation.

Results

Sister centromeres frequently become dissociated and remain between the spindle poles during anaphase I in *rec12* mutant cells

Elimination of chiasmata induced by depletion of Rec12, a recombination factor required for the formation of double-strand breaks [36], causes occasional equational segregation of sister chromatids [33] and frequent non-disjunction of homologous chromosomes [37]. As a first step toward understanding the role of chiasmata in the spindle attachment of sister chromatids, we re-examined chromosome segregation during meiosis I in more detail in *rec12* mutant cells.

We examined chromosome segregation by visualizing centromere-linked loci of chromosome I (the *lys1* locus: *cen1*) and chromosome II (the *D107* locus: *cen2*) using green fluorescent protein (GFP) [33]. After the first division, homologous centromeres were partitioned into two nuclei and rarely into the same nucleus in wild-type cells (Figure 2A). In contrast, homologous centromeres were frequently partitioned into the same nucleus in *rec12* mutant cells (Figure 2A). Furthermore, sister centromeres were partitioned into the same nucleus and rarely into two nuclei in wild-type cells but were occasionally partitioned into two nuclei in *rec12* mutant cells (Figure 2B, +, *rec*+ and *rec12Δ*). The ~4% of wild-type cells that showed a partition of *cen1* into the distinct nuclei was most likely the result of recombination between the centromere and the *lys1* locus used for this analysis. These results confirmed the mis-segregation of both homologous chromosomes and sister chromatids during meiosis I in *rec12* mutant cells. The same mis-segregation phenotypes were also observed in cells lacking the Rec14 recombination factor, which functions together with Rec12 and the depletion of which eliminates recombination (Figure 2A and 2B) [38,39].

Segregation analysis showed that the overall mis-segregation frequency of sister chromatids in recombination-deficient, chiasmata-lacking cells (i.e., achiasmate cells) was small. However, live cell analysis of *cen2* dynamics suggested that improper spindle attachment of sister centromeres occurs more frequently during anaphase I. Although the sister centromeres eventually moved to the pole in *rec12* mutant cells, they frequently remained between the two spindle poles and were dissociated during anaphase I [observed for 7 out of 14 centromeres examined (50.0%); Figure 2C, *rec12Δ*]. These centromeres are called lagging centromeres, and they were not observed in wild-type cells [observed for 0 of 12 centromeres examined (0%); Figure 2C, Wt]. The chromosome lagging is most likely caused by a loss of chiasmata and not a loss of Rec12 function, because lagging chromosomes were also frequently observed when meiosis was induced in haploid cells [33], which do not form chiasmata due to their lack of homologous chromosomes (Figure S1). These results suggest that sister centromeres are frequently attached to both poles and are pulled from opposite directions during anaphase I in achiasmate cells.

Sgo1 depletion causes equational segregation of sister chromatids during meiosis I in achiasmate cells

To confirm the frequent bipolar attachment of sister chromatids in achiasmate cells, we depleted Sgo1, which inhibits the removal of centromeric cohesin during anaphase I [17,19]. We hypothesized that although sister chromatids are frequently attached to both poles and are pulled from opposite directions, the centromere cohesion that persists until meiosis II should provide resistance against this force and prevent their separation during anaphase I in achiasmate cells. If so, depletion of Sgo1, which eliminates centromere cohesion during anaphase I, should lead to frequent equational segregation of sister chromatids.

Indeed, Sgo1 depletion led to a substantial increase in equational segregation in achiasmate cells. Equational segregation of sister centromeres was occasionally observed in *sgo1* mutant cells but was more frequently observed in *sgo1 rec12* and *sgo1 rec14* double-mutant cells (Figure 2B, *sgo1Δ*). When meiosis I was induced in haploid cells, Sgo1 depletion similarly increased equational segregation (Figure 2D) irrespective of Rec12 depletion (data not shown). Therefore, the increased equational segregation is not specific to recombination-deficient cells but is common in achiasmate cells. These results confirm that the loss of chiasmata frequently leads to the bipolar attachment of sister chromatids during anaphase I.

Bipolar attachment of sister chromatids only partially depends on the spindle assembly checkpoint (SAC) in achiasmate cells

The SAC ensures faithful chromosome segregation by delaying anaphase initiation until all of the chromosomes become properly attached to the spindle [40,41]. We previously reported that the SAC becomes activated to delay anaphase initiation at meiosis I in *rec12* mutant cells, which is likely associated with improper spindle attachment of chromosomes [42]. Similarly, analysis of spindle length showed that anaphase initiation was substantially delayed in *sgo1 rec12* double-mutant cells in a Mad2-dependent manner, as previously observed in *rec12* mutant cells (Figure S2A, Text S1). Therefore, we next examined whether the SAC contributes to the bipolar attachment of sister chromatids in achiasmate cells by depleting the SAC factor Mad2.

Mad2 depletion led to decreased equational segregation of sister chromatids in *rec12* mutant cells, but equational segregation was still observed at substantial levels in *rec12 sgo1* double-mutant cells (Figure 3A). Likewise, Mad2 depletion decreased but did not abolish equational segregation during meiosis I in haploid cells (Figure 3B). These results showed that the SAC promotes the bipolar attachment of sister chromatids but is not essential for this process in achiasmate cells. Furthermore, as seen in *rec12* mutant cells, sister centromeres frequently dissociated and failed to move to the pole during anaphase I in *mad2 rec12* double-mutant cells [40.9% (22 centromeres);

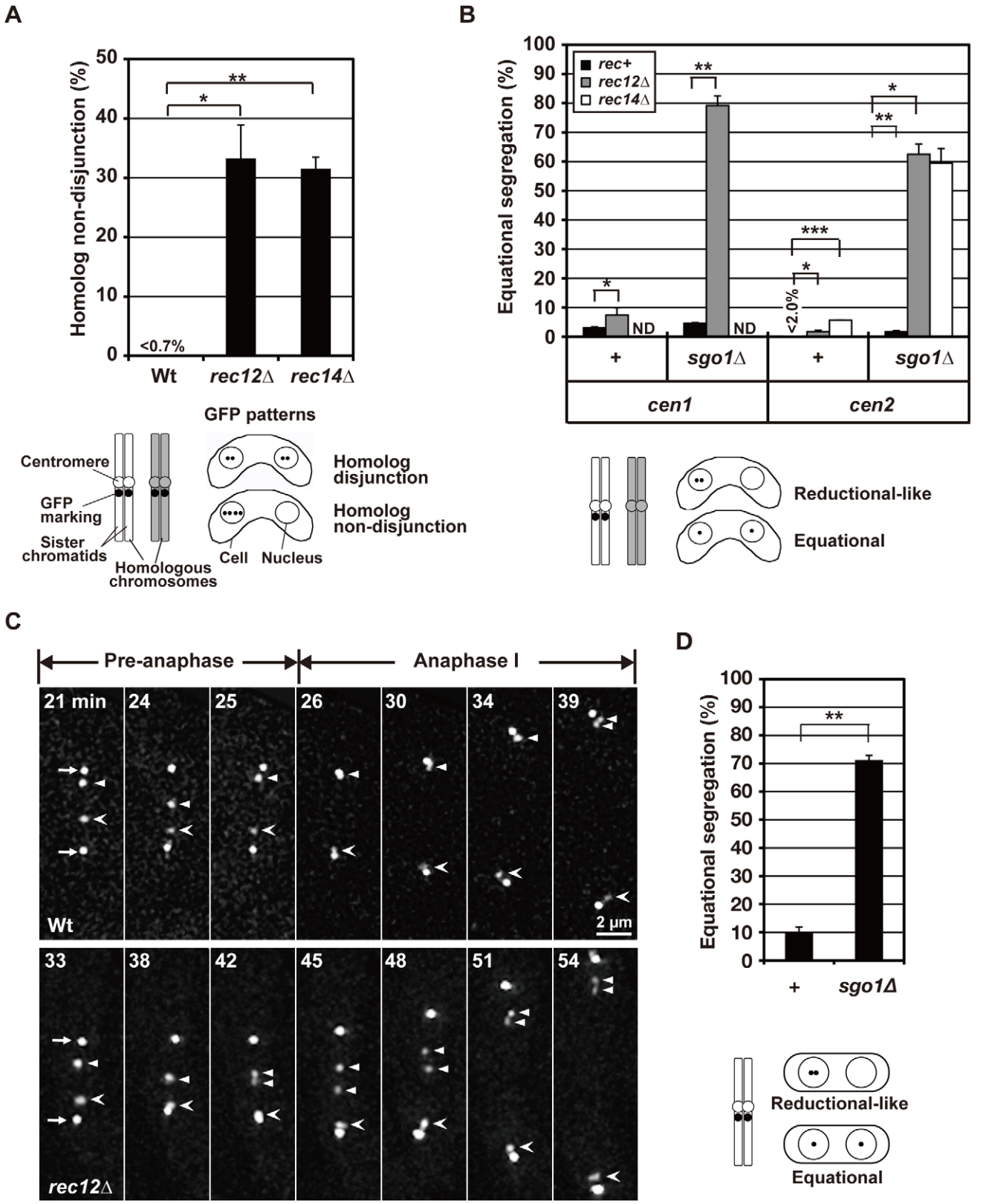

Figure 2. The effect of loss of chiasmata on chromosome segregation. (A) The frequency of non-disjunction of homologous chromosomes during meiosis I was examined by GFP-visualized *cen2*. (B) Equational segregation of sister chromatids during meiosis I in various types of diploid cells. +: no *sgo1* mutation; *cen1*: the *lys1* locus; *cen2*: the *D107* locus [33]; *rec+*: no *rec12* or *rec14* mutation. ND: not determined. (C) Centromere dynamics in wild-type and *rec12* mutant cells during meiosis I. Arrows indicate the spindle pole bodies (SPBs). Arrowheads and barbed arrowheads show homologous centromeres (*cen2*). Pre-anaphase: the pre-anaphase stage, as determined by a constant pole-to-pole distance. Anaphase I: anaphase I, as determined by an increase in the pole-to-pole distance. Numbers indicate the time in minutes from the beginning of spindle

formation. (D) Equational segregation of sister chromatids at meiosis I induced in haploid cells. Sister chromatid segregation was analyzed using GFP-visualized *cen2*. +: *sgo1*$^{+}$ cells; *sgo1Δ*: *sgo1* mutant cells. The lower illustrations in (A), (B), and (D) show how GFP was used to mark chromosomes (left) and the segregation patterns of the GFP signals after meiosis I (right). In all analyses in this study, with the exception of analyses in the supplementary results, sister chromatid segregation was analyzed in cells containing two DNA masses that underwent meiosis I. Each data point was obtained from two independent experiments, with the exception of the non-disjunction frequency of homologous chromosome in *rec12* mutant cells, which was obtained from three independent experiments. More than 50 cells were examined in each experiment. Error bars indicate standard deviation. Asterisks indicate statistically significant differences and their associated *p* values, as determined by t-tests. *$p<0.05$; ** $p<0.005$; *** $p<5\times10^{-5}$.
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Figure 3C, *mad2Δ rec12Δ*]. However, lagging centromeres were rarely observed in *mad2* mutant cells [0.1% (20 centromeres); Figure 3C, *mad2Δ*], although the timing of anaphase initiation was not much different between these mutants (Figure S2A). These results indicated that the lagging centromeres seen in achiasmate cells were not caused by SAC activation or delayed anaphase initiation. Thus, we conclude that the bipolar attachment of sister chromatids depends only partially on the SAC in achiasmate cells.

Bipolar attachment of sister chromatids occasionally occurs before anaphase irrespective of chiasma formation

Spindle attachment of chromosomes is established before anaphase, and the chiasma may prevent the bipolar attachment of sister chromatids from occurring during the pre-anaphase stage. To test this possibility, we examined the dynamics of sister centromeres before anaphase by time-lapse analysis with 10-s intervals. The time-lapse analysis of *cen2* loci on both homologous chromosomes in wild-type and *rec12* mutant cells confirmed our previous observations from time-lapse analyses with 1-min intervals, although they exhibited slight differences in dynamic parameters (Table S1) [42]. Homologous centromeres oscillated between the two spindle poles in a somewhat coordinated manner in wild-type cells; a pair of homologous centromeres often moved in the same direction (Figure 4A, Table S2). Accordingly, centromeres were mostly positioned around the middle point between the spindle pole and the spindle center with a tendency to be near the center (Figure 4B). These centromere dynamics presumably reflect the frequent bipolar attachment of homologous chromosomes that are linked by the chiasmata (Figure 4C). On the other hand, sister centromeres oscillated in an uncoordinated manner and tended to remain near the pole in *rec12* mutant cells (Figure 4A), and centromere positioning was shifted toward the pole (Figure 4B). These centromere dynamics probably reflect the frequent attachment of each of the non-linked homologous chromosomes to one pole and the occasional switch in their attachment to the other pole (Figure 4C).

Notably, we found that sister centromeres occasionally underwent a transient dissociation in both wild-type and *rec12* mutant cells (Figure 4A and 4D, Table 1). In both types of cells, centromere dissociation was observed in ~20% of events on average (Figure 4E). This dissociation was not the result of the integration into the chromosome of lacO repeats, which are used for visualization [33], or of the dissociation of only the visualized pericentromeric region; when all three homologous sets of sister centromeres were visualized by GFP tagging of the centromere-specific histone H3 variant Cnp1 [43], we observed more than six centromere signals together with a transient split of the signal into two (Figure 4F). These observations showed that bipolar attachment of sister chromatids occasionally occurs during the pre-anaphase stage, irrespective of chiasma formation. Similar centromere dynamics were also observed in cells lacking Sgo1. The occurrence of bipolar attachment in the presence of chiasmata is contradictory to the idea that chiasmata prevent the bipolar attachment of sister chromatids from occurring during the pre-anaphase stage.

Sister chromatids attach to both poles more frequently in *mrc1 rec12* and *moa1 rec12* double-mutant cells than in *rec12* single-mutant cells

If chiasmata do not prevent the bipolar attachment of sister chromatids from occurring, they must contribute to the elimination of bipolar attachment of sister chromatids during the pre-anaphase stage. However, the overall frequency of centromere dissociation was not significantly different between wild-type and *rec12* mutant cells (Figure 4E, Table 1), and chiasma-dependent elimination of the bipolar attachment was not evident. We hypothesized that if sister centromeres attach to both poles more frequently in the achiasmate background, the chiasma-dependent elimination of the bipolar attachment would be evident. Following this hypothesis, we examined *mrc1* and *moa1* mutant cells.

The *mrc1* gene encodes a conserved DNA replication checkpoint factor, which delays cell cycle progression upon DNA replication stress, promotes proper fork progression, and contributes to sister chromatid cohesion in mitosis [44–50]. On the other hand, the *moa1* gene encodes a meiosis-specific centromere protein that contributes to the proper centromere localization of the meiotic cohesin component Rec8 [34]. In both *mrc1* and *moa1* mutant cells, chromosome segregation as well as spindle dynamics, recombination, and spore formation are largely normal (Figures S2B and S3, Text S1) [34]. However, sister chromatids are primarily segregated equationally in a manner partly dependent on Mad2 when chiasmata are not formed (in the *rec12Δ* or the haploid background; Figure 5) [34]. Although these phenotypes are similar to the *sgo1*-mutant phenotypes, the equational segregation is primarily caused by defects in centromere features other than maintenance of centromere cohesion, because both *mrc1* and *moa1* mutant cells can maintain sister centromere cohesion until anaphase II if sister chromatids are not segregated equationally during meiosis I (Figure S3D, Text S1) [34]. Therefore, the equational segregation seen in the *mrc1 rec12* and *moa1 rec12* mutant cells is likely to be caused by frequent bipolar attachment of sister chromatids, and we expected that the chiasma effects would be more evident in the *mrc1* and *moa1* mutants.

To evaluate chiasma effects in the *mrc1* and *moa1* mutants, we first examined the pre-anaphase centromere dynamics in the achiasmate *mrc1 rec12* and *moa1 rec12* double-mutant cells. In the *mrc1 rec12* mutant cells, the sister centromeres dissociated more frequently (Figure 6A and 6B), with a significantly longer duration (Table 1), and were predominantly positioned around the spindle center, unlike those in the *rec12* mutant cells (Figure 6C). In the *moa1 rec12* mutant cells, the centromeres were also frequently positioned around the spindle center (Figure 6A and 6C), and in addition, the SAC was not activated as much as in *rec12* mutant cells (Figure S2A, Text S1). These characteristics were expected to be associated with frequent bipolar attachment of sister chromatids (Figure 6D). Indeed, the frequent dissociation of the centromeres and their positioning

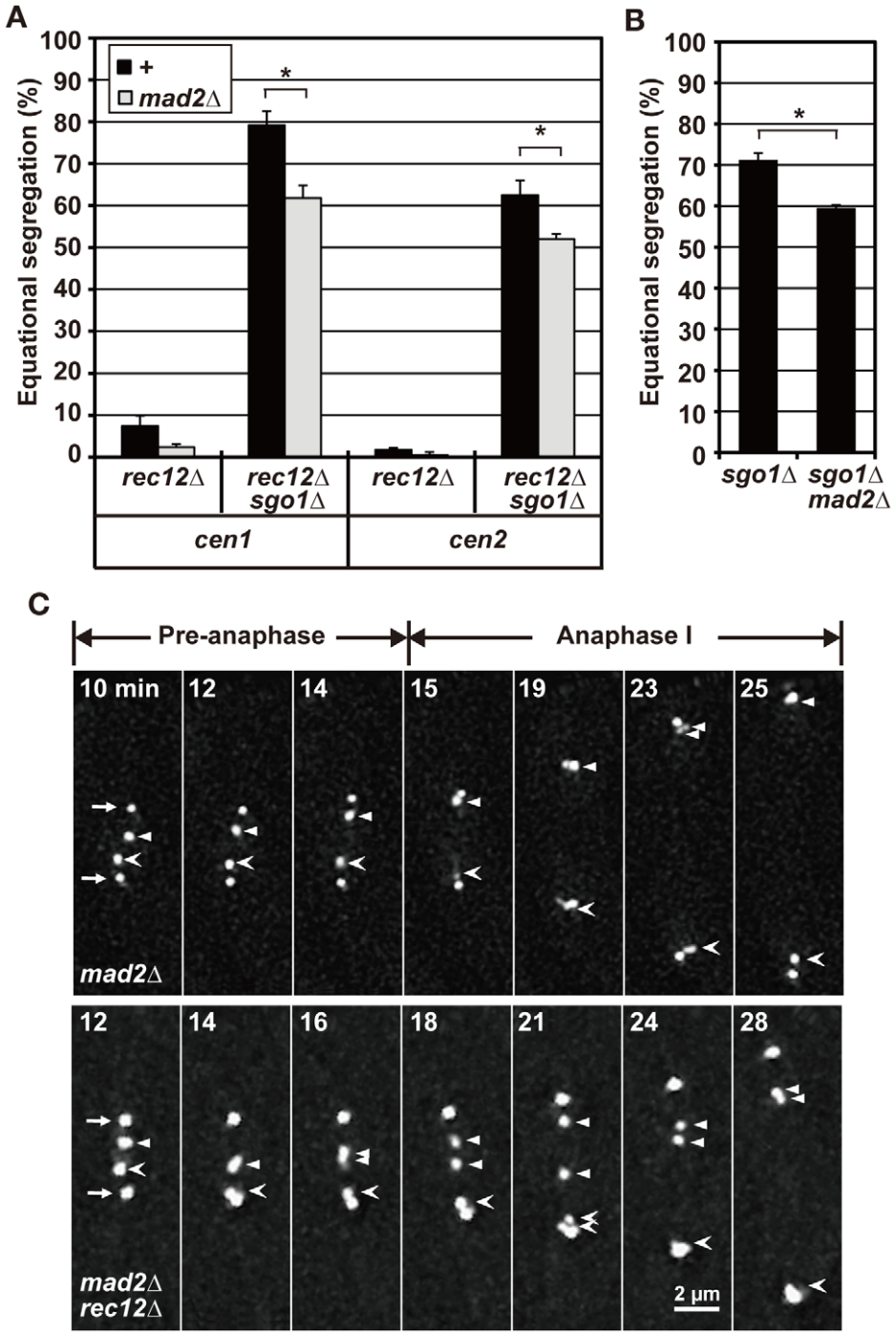

Figure 3. A role for the SAC factor, Mad2, in chromosome segregation during meiosis I. (A) Effects of Mad2 depletion on sister chromatid segregation during meiosis I in *rec12* achiasmate cells. +: no *mad2* mutation. (B) Effects of Mad2 depletion on sister chromatid segregation at meiosis I in haploid cells. Sister chromatid segregation was analyzed using GFP-visualized *cen2*. (C) Effects of Mad2 depletion on centromere dynamics during meiosis I. Arrows indicate the SPBs. Arrowheads and barbed arrowheads show each of the homologous centromeres (*cen2*). Numbers indicate the time in minutes from the beginning of spindle formation. Bar: 2 μm. In (A) and (B), each value was obtained from two independent experiments, with the exception of the equational frequencies for *rec12 sgo1 mad2* cells, which were obtained from three independent experiments. Error bars indicate standard deviation. Asterisks show statistically significant differences ($p<0.05$).
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around the spindle center together with the low level of SAC activation were observed during meiosis I in achiasmate *rec8* mutant cells (Figure 6A–6C and Figure S2A, Table 1), in which sister chromatids efficiently attach to both poles to fully undergo equational segregation [12,51]. They were also observed during mitotic division in wild-type diploid cells (Figure S4). These observations thus confirmed that sister centromeres attach to both poles more frequently in the *mrc1 rec12* and *moa1 rec12* double-mutant cells than in *rec12* single-mutant cells. However, the centromere properties of the *mrc1* and *moa1* mutant cells

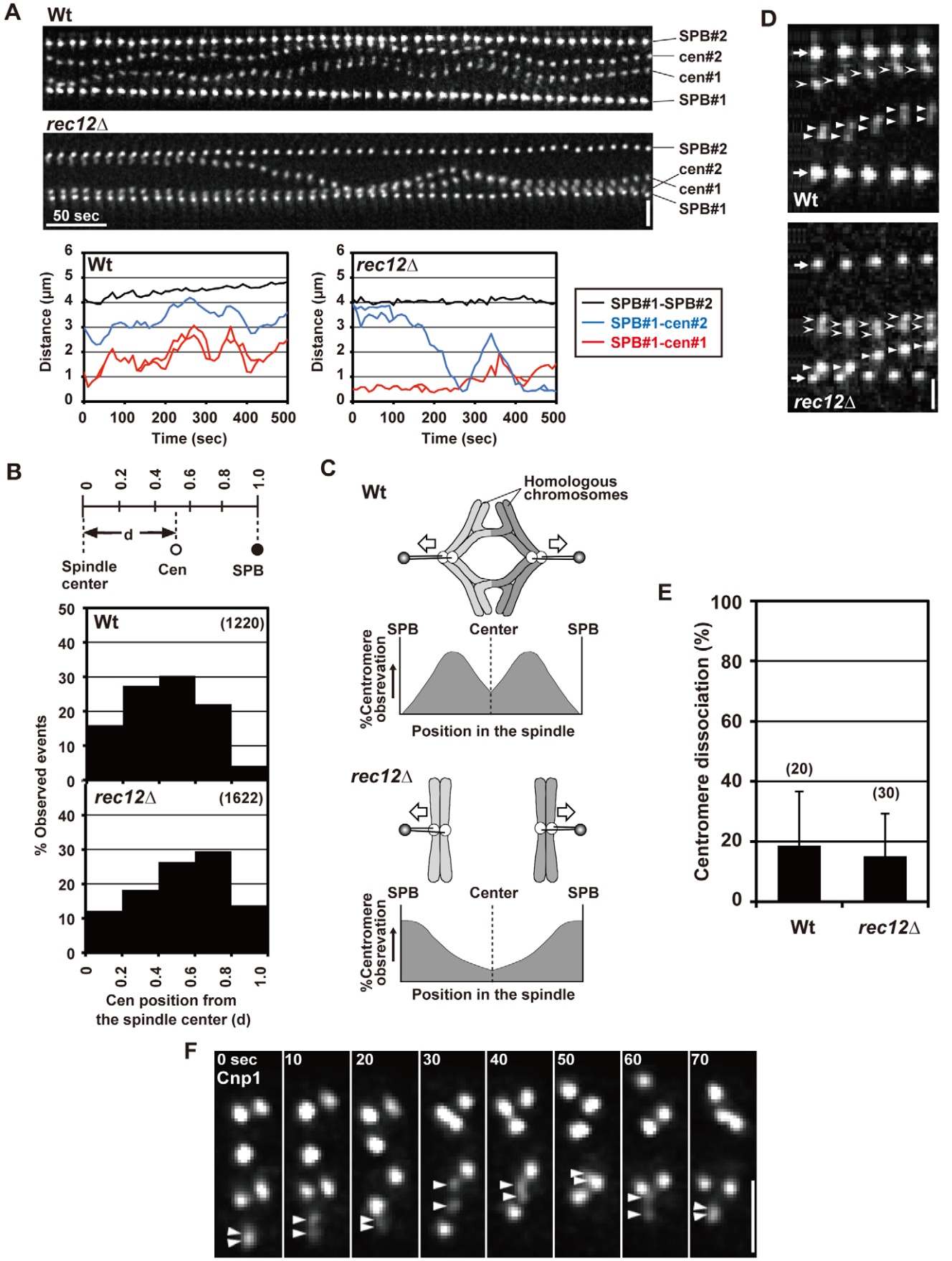
A
Wt
SPB#2
cen#2
cen#1
SPB#1
rec12Δ
SPB#2
cen#2
cen#1
SPB#1
50 sec
Wt
rec12Δ
Distance (μm)
Time (sec)
SPB#1-SPB#2
SPB#1-cen#2
SPB#1-cen#1
D
Wt
rec12Δ
B
Spindle center
Cen
SPB
d
Wt (1220)
rec12Δ (1622)
% Observed events
Cen position from the spindle center (d)
C
Wt
Homologous chromosomes
SPB
Center
SPB
%Centromere obsrevation
Position in the spindle
rec12Δ
SPB
Center
SPB
%Centromere obsrevation
Position in the spindle
E
Centromere dissociation (%)
(20)
(30)
Wt
rec12Δ
F
0 sec
Cnp1
10
20
30
40
50
60
70

Figure 4. Pre-anaphase centromere dynamics during meiosis I in wild-type and *rec12* mutant cells. (A) Pre-anaphase dynamics of the spindle pole and centromere (*cen2*) during meiosis I. Photos were taken every 10 s and are shown in order from left to right. Horizontal bar: 50 s. Vertical bar: 2 μm. Graphs show changes in the distance between the SPB and each centromere (red, SPB#1-cen#1, and blue, SPB#1-cen#2) and between the two SPBs (black, SPB#1-SPB#2). (B) Observation frequencies of centromeres at distinct positions in the spindle during the pre-anaphase stage. The positions of centromeres are shown as relative distances from the spindle center (d), as determined in the upper illustration. Zero and 1.0 correspond to positions of the spindle center and the SPB, respectively. The number of examined positions is shown in parentheses. (C) Spindle attachment of chromosomes during meiosis I in wild-type (Wt) and *rec12* mutants (*rec12Δ*) and expected observation frequencies of centromeres at distinct positions in the spindle. For *rec12* mutant, only the attachment of homologous chromosomes to both poles is shown. (D) Dissociation of GFP-visualized sister centromeres (*cen2*). Arrows indicate the SPB. Arrowheads and barbed arrowheads show each of the homologous centromeres. Bar: 1 μm. (E) Average dissociation frequencies of sister centromeres in wild-type and *rec12* mutant cells. The number of centromeres examined is shown in parentheses. (F) Dissociation of sister centromeres visualized by GFP-tagged Cnp1 during the pre-anaphase stage. The stage was determined based on centromere behavior and the distance between the spindle poles visualized using the DsRed-tagged SPB component Sad1 (not shown). Arrowheads indicate sister centromeres that underwent dissociation. Numbers at the top indicate the time in seconds. Bar: 1 μm. In analyses of centromere position and dissociation, 20 and 28 pairs of sister centromeres were examined for wild-type and *rec12* mutant strains, respectively. More than 10 time points were examined for each centromere analysis. Error bars indicate standard deviation.
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differed from those of *rec8* mutant or mitotic cells because the SAC substantially delayed anaphase initiation in *mrc1 rec12* mutant cells (Figure S2A, Text S1), and centromere dissociation was not so frequent in *moa1 rec12* mutant cells (Figure 6B).

Chiasmata prevent the bipolar attachment of sister chromatids in *mrc1* mutant cells but not in *moa1* mutant cells

We next examined the pre-anaphase centromere dynamics in the chiasmate *mrc1* and *moa1* single-mutant cells to evaluate chiasma effects. Remarkably, in *mrc1* single-mutant cells, the level of centromere dissociation was almost identical to that in wild-type cells (Figure 6A and 6B, Table 1), indicating that bipolar attachment of sister chromatids was reduced to a wild-type level. Furthermore, centromere positioning and the distance between homologous centromeres were very similar to what was seen in wild-type cells (Figure 6C and 6E), indicating that homologous chromosomes attach to both poles as frequently as in wild-type cells. These results show that chiasmata eliminate the bipolar attachment of sister chromatids and promote the bipolar attachment of homologous chromosomes during the pre-anaphase stage in *mrc1* mutant cells.

On the other hand, in *moa1* mutant cells, centromere positioning and dissociation were not significantly different from those seen in achiasmate *moa1 rec12* mutant cells (Figure 6A–6C, Table 1). Furthermore, homologous centromeres were not separated as widely as in wild-type cells (Figure 6E). These results indicate that sister chromatids still attach to both poles at a level similar to that in *moa1 rec12* mutant cells and pulling forces are not properly exerted on homologous chromosomes in *moa1* mutant cells (Figure 6E). Therefore, chiasmata fail to eliminate the bipolar attachment of sister chromatids during the pre-anaphase stage in *moa1* mutant cells.

Chiasmata induce the preferential exertion of segregation forces on sister chromatids toward the proper pole during anaphase I in *moa1* mutant cells

Because the bipolar attachment of sister centromeres did not appear to be eliminated during the pre-anaphase stage in chiasmate *moa1* mutant cells, we examined whether their bipolar attachment is retained during anaphase by analyzing anaphase centromere dynamics. In wild-type cells, sister centromeres moved swiftly toward the poles (all 13 of the centromeres examined reached the poles within 130 s; Figure 7) and only occasionally dissociated during anaphase I [only three centromeres out of 13 (23.1%) were dissociated; Figure 7, Wt, lower panel]. The centromeres also moved swiftly to the pole and remained associated in *mrc1* mutant cells (all 11 centromeres examined reached the pole within 80 s without dissociation; Figure 7). In contrast, in *moa1* mutant cells, lagging and dissociation of centromeres were frequently observed during anaphase [10 out of 14 centromeres (71.4%) failed to reach the poles within 130 s, unlike wild-type centromeres, and 5 of them (35.7%) failed to reach the poles within 300 s; 6 centromeres (42.9%) were dissociated; Figure 7]. Furthermore, elimination of anaphase centromere cohesion by Sgo1 deletion substantially increased the equational segregation of sister chromatids (Figure 5B). These results showed that sister chromatids were frequently attached to both poles and pulled from opposite directions during anaphase I in *moa1* mutant cells. Surprisingly, most of the lagging centromeres eventually moved to the proper pole (Figure 5A and 5B, Figure 7). This result indicates that although sister chromatids were pulled from opposite directions during anaphase, they were pulled toward the proper pole more strongly and/or continuously than they were pulled toward the improper pole in the chiasmate *moa1* mutant cells. Therefore, the chiasma generates a bias toward the proper

Table 1. Centromere dissociation during the pre-anaphase stage of meiosis I in various fission yeast strains.

Strain	Duration¶ (sec)	Observation§ (%)	Total number of time points examined
Wt	27.2±26.4	19.4	1,026
rec12	25.1±27.2	18.0	1,492
mrc1	21.1±18.8	18.8	933
mrc1 rec12	48.2±52.8	52.7	465
moa1	24.5±19.4	18.6	1,620
moa1 rec12	26.8±21.0	17.4	1,082
rec8	40.7±38.9	35.4	585

¶Duration: average duration of centromere dissociation ± standard deviation.
§Observation: percentages of observed dissociation events.
doi:10.1371/journal.pgen.1001329.t001

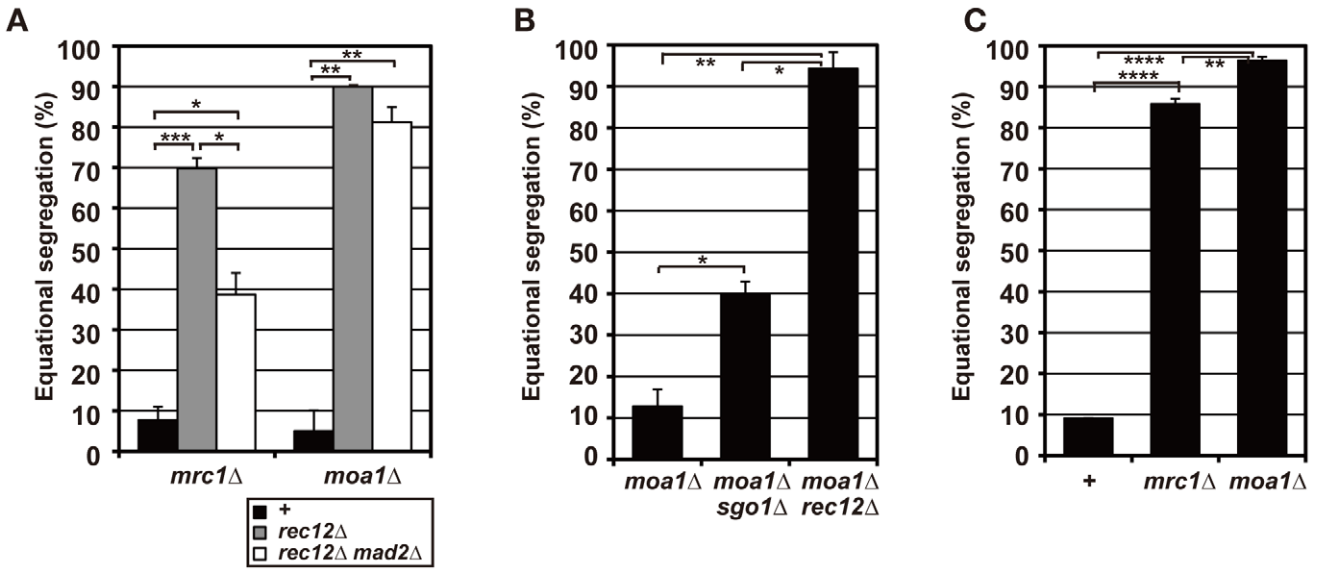

Figure 5. Sister chromatid segregation in *mrc1* and *moa1* mutants. (A) Sister chromatid segregation in *mrc1* and *moa1* mutants and the effects of Rec12 or Mad2 depletion analyzed by the GFP-visualized *cen2*. +: no *rec12* or *mad2* mutation. (B) Sister chromatid segregation in *moa1* mutant and the effects of Sgo1 or Rec12 depletion analyzed by the GFP-visualized *cen1*. (C) Effects of Mrc1 or Moa1 depletion on sister chromatid segregation at meiosis I in haploid cells. Sister chromatid segregation was analyzed by the GFP-visualized *cen2*. Data values in all graphs were obtained as described in Figure 2. Error bars indicate standard deviation. Asterisks show statistically significant differences and their associated *p* values. * $p<0.05$; ** $p<0.005$; *** $p<0.0005$; **** $p<5\times10^{-6}$.
doi:10.1371/journal.pgen.1001329.g005

pole in poleward chromosome pulling from opposite directions that eventually results in proper chromosome segregation in *moa1* mutant cells.

Discussion

Chiasmata play a crucial role in preventing the bipolar attachment of sister chromatids during anaphase I

In the current study, we examined the role of chiasmata by analyzing the segregation and dynamics of chromosomes during meiosis I induced in recombination-deficient diploid cells and in haploid cells. The analysis of these two distinct types of achiasmate cells provided two lines of evidence to show that sister chromatids frequently attach to both poles and experience pulling forces from opposite directions during anaphase I in achiasmate cells. First, sister centromeres frequently became transiently dissociated and/or failed to move to the pole during anaphase I (Figure 2C and Figure S1). Second, when sister centromere cohesion was resolved during anaphase by Sgo1 depletion, sister chromatids frequently underwent equational segregation during anaphase I (Figure 2B and 2D). Chiasmata therefore play a crucial role in preventing the bipolar attachment of sister chromatids during anaphase I. Because the bipolar attachment of sister chromatids has been observed during anaphase I in various achiasmate organisms [27–29], it is probably common among eukaryotes.

Two distinct tasks of chiasmata: elimination of the bipolar attachment of sister chromatids and induction of a bias in poleward chromosome pulling

We further examined how chiasmata prevent the bipolar attachment of sister chromatids. Loss of chiasmata causes activation of the SAC [42]. However, we showed that the bipolar attachment of sister chromatids depends only partially on the SAC in achiasmate cells. The reduction of the bipolar attachment that normally generates tension in the achiasmate background is consistent with the idea that the SAC promotes attachments that generate tension [40,41].

We performed high time-resolution analysis of pre-anaphase centromere dynamics in several different types of chiasmate and achiasmate cells to understand how chiasmata contribute to the attachment. From this analysis, we have reached three conclusions. First, chiasmata cannot prevent occurrence of bipolar attachment of sister chromatids, based on the observation that the bipolar attachment occasionally occurred in chiasmate wild-type cells.

Second, analysis of *mrc1* mutant cells showed that chiasmata contribute to the elimination of the bipolar attachment of sister chromatids during the pre-anaphase stage (Figure 8A). However, the elimination was not evident in wild-type cells in comparison with *rec12* mutant cells. One possible explanation for this result is that the bipolar attachments occur more frequently in wild-type than in *rec12* mutant cells because the centromere is positioned closer to the spindle center in wild-type cells (Figure 4B). Alternatively, chiasmata may eliminate bipolar attachments in *mrc1* mutant cells but not in wild-type cells because of distinct centromere structures or functions. Furthermore, we cannot completely exclude the possibility that the chiasmata-dependent elimination depends in part on unknown Rec12 functions.

Third, analysis of *moa1* mutant cells showed that chiasmata induced a bias toward the proper pole in poleward chromosome pulling from opposite directions that resulted in proper chromosome segregation (Figure 8B). In *moa1* mutant cells, sister centromeres were frequently pulled from opposite directions and dissociated during anaphase I, but they were pulled toward the proper pole more strongly and/or continuously than they were pulled toward the improper pole, and eventually moved to the appropriate pole. We also observed this chiasma effect, albeit occasionally, in wild-type cells (Figure 7, Wt, lower panel) and thereby speculate that the chiasma-induced bias is a backup mechanism that ensures proper meiotic chromosome segregation even when improper attachments remain.

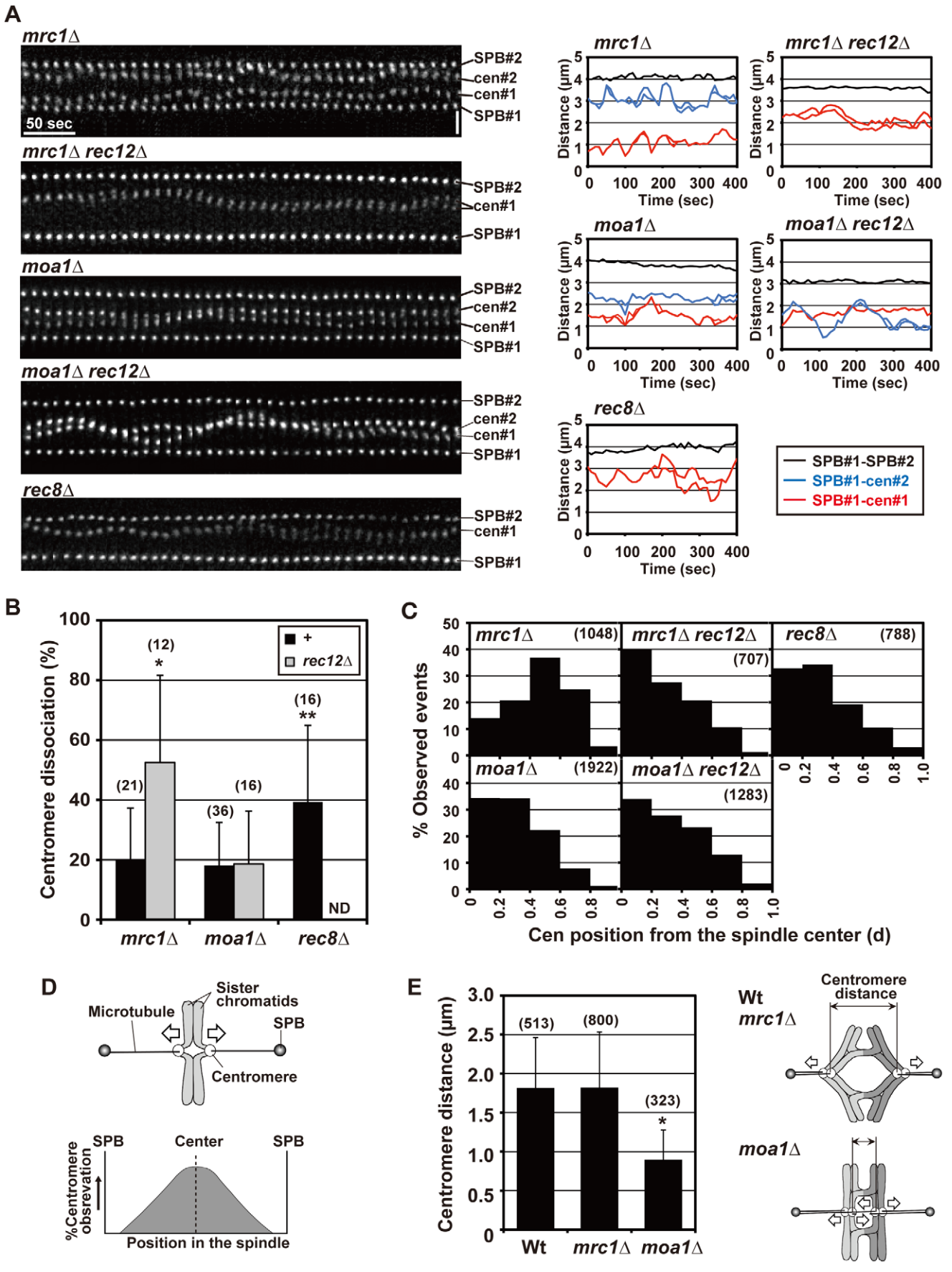
A
mrc1Δ
SPB#2
cen#2
cen#1
SPB#1
50 sec
mrc1Δ rec12Δ
moa1Δ
moa1Δ rec12Δ
rec8Δ
Distance (μm)
Time (sec)
SPB#1-SPB#2
SPB#1-cen#2
SPB#1-cen#1
B
Centromere dissociation (%)
+
rec12Δ
(12)
(16)
(21)
(16)
(36)
ND
C
% Observed events
(1048)
(707)
(788)
(1922)
(1283)
Cen position from the spindle center (d)
D
Sister chromatids
Microtubule
SPB
Centromere
%Centromere obsrevation
Center
Position in the spindle
E
Centromere distance (μm)
(513)
(800)
(323)
Wt
Centromere distance

Figure 6. Pre-anaphase centromere dynamics during meiosis I in *mrc1* and *moa1* mutants. (A) Pre-anaphase dynamics of the spindle pole and centromere (*cen2*) at meiosis I, and changes in the distance between the spindle pole and the centromere and between the two spindle poles in *mrc1*, *moa1*, and *rec8* mutants. Note that only one of the homologous centromeres is visualized in *mrc1 rec12* and *rec8* mutant cells. Horizontal bar: 50 s. Vertical bar: 2 μm. (B) Average centromere dissociation frequencies in *mrc1*, *moa1*, and *rec8* mutant cells. The number of centromeres examined is shown in parentheses. +: no *rec12* mutation. ND: not determined. Asterisks indicate dissociation frequencies that are statistically different from the frequency of wild type. * $p<0.005$; ** $p<0.01$. (C) Observation frequencies of centromeres at distinct positions in the spindle during the pre-anaphase stage. The positions of centromeres are shown based on their relative distance from the spindle center (d), as determined in Figure 4B. The number of examined positions is shown in parentheses. (D) Bipolar attachment of sister chromatids and expected observation frequencies of centromeres at distinct positions in the spindle. (E) Distance between homologous centromeres. The distance between homologous centromeres was measured at every time point in each strain, and an average distance is shown. When centromeres were dissociated, the distance between the nearest homologous pair of centromeres was measured. The asterisk indicates a distance statistically different from that of wild type ($p<5\times10^{-125}$). The number of distances examined is shown in parentheses. Right illustrations show models for spindle attachment of chromosomes and the resultant distance between the centromeres in wild-type, *mrc1*, and *moa1* mutant cells. White arrows in all illustrations indicate forces exerted on chromosomes. Error bars in all graphs indicate standard deviations.
doi:10.1371/journal.pgen.1001329.g006

Mechanism associated with chiasma-dependent elimination of bipolar attachments and biased chromosome pulling

How the chiasmata eliminate bipolar attachments and induce a bias in chromosome pulling remains elusive. Because chiasmata are essential for generating the tension that stabilizes kinetochore–microtubule interactions and increases kinetochore microtubules [9,52], we speculate that chiasmata execute these different tasks via tension, as follows (see also Text S1). In wild-type cells, sister kinetochores occasionally attach to both poles (Figure S5A). In the presence of chiasmata, microtubules that attach to the proper poles generate sufficient tension, but those that attach to improper poles probably do not. As a result, improper attachments are eliminated while proper attachments are increased. Even when improper attachments are not eliminated, the increase in proper attachments presumably promotes the exertion of segregation forces in the appropriate direction (a similar scenario is shown in Figure S5A, *rec12Δ*). In contrast, improper attachments are not eliminated in *rec12* mutant cells, possibly because the improper attachments also generate tension (Figure S5A).

In this model, chiasmata must prevent improper attachments from generating tension. During the pre-anaphase stage, chromosomes oscillate between the poles, and oscillation of the chiasma-linked chromosomes may reduce tension (Figure S5B). When a pair of sister chromatids follows the other homologous pair that is moving toward the spindle pole, the leading sister chromatid pair presumably exerts pulling forces on the chromosome arms of the following pair via chiasmata. These pulling forces are likely to reduce the tension that improper attachments generate but not those generated by proper attachments. As a result, only proper attachments (i.e., bipolar attachment of the homologous chromosomes) become stable and persist, whereas improper attachments (i.e., bipolar attachment of sister chromatids) do not. Alternatively, the chiasmata-dependent pulling may make the kinetochores on the following chromosomes face the side opposite the direction of chromosome movement to physically eliminate improper attachments. Although the above model can account for the observed chiasmata-dependent effects, we cannot completely rule out the possibility that chiasmata directly contribute to centromere function or structure to affect spindle attachment and segregation of chromosomes.

Effects of kinetochore arrangement on the chiasma-dependent elimination of improper attachments

Chiasmata eliminated bipolar attachment of sister chromatids in the *mrc1* mutant but did not eliminate it in the *moa1* mutant. Distinct kinetochore arrangements may account for this difference

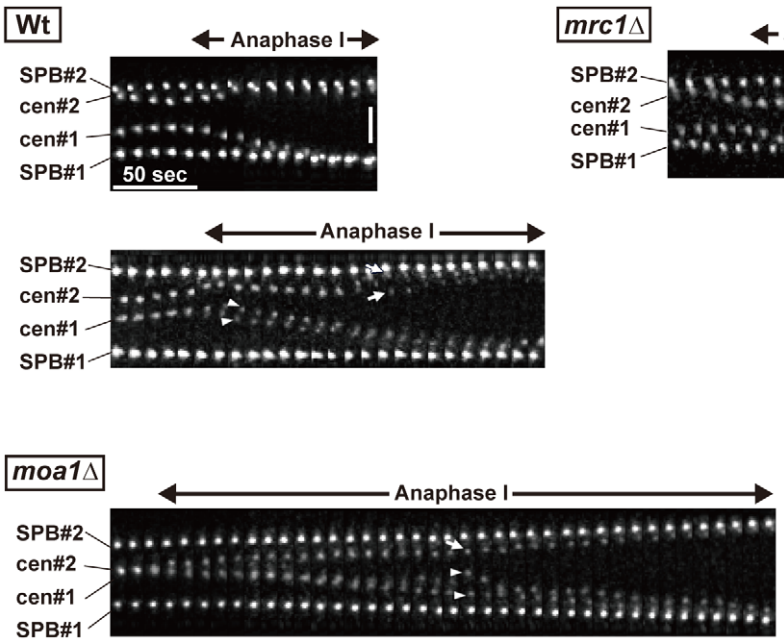

Figure 7. Centromere dynamics during anaphase I in *mrc1* and *moa1* mutants. Arrows and arrowheads show each of the homologous centromeres (*cen2*), respectively, and the two arrowheads or arrows indicate dissociated sister centromeres. Horizontal bar: 50 s. Vertical bar: 2 μm.
doi:10.1371/journal.pgen.1001329.g007

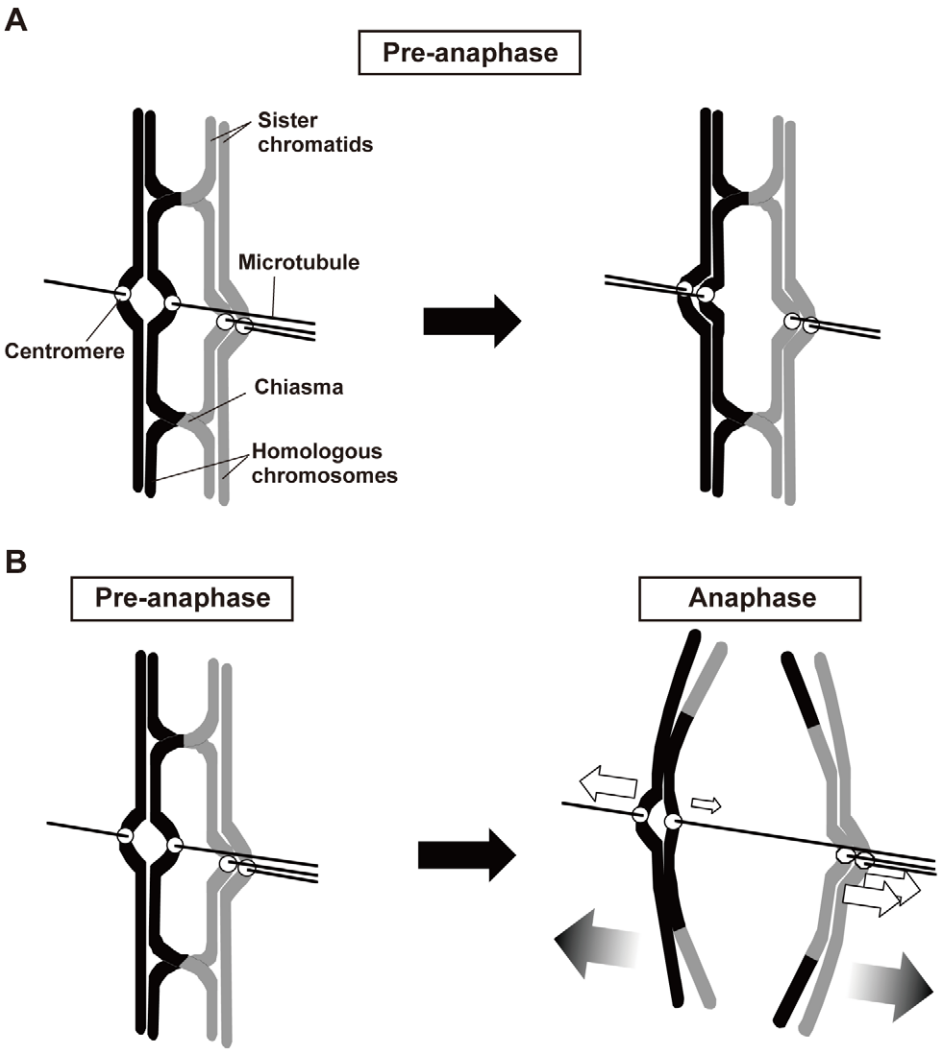

Figure 8. Two major roles of chiasmata during meiosis I. (A) Chiasmata eliminate the bipolar attachment of sister centromeres (centromeres on left sister chromatids) during the pre-anaphase stage of meiosis I. (B) When the bipolar attachment remains during anaphase, chiasmata generate bias in the poleward pulling forces to cause proper chromosome segregation. White arrows indicate the pulling forces exerted on chromosomes during anaphase I. A smaller arrow indicates a weaker or less continuously exerted force. For simplicity, only a single microtubule is shown to illustrate the spindle attachment of each kinetochore.
doi:10.1371/journal.pgen.1001329.g008

(Figure S5A, Text S1). Given the frequent monopolar attachment of sister chromatids in the chiasmate *mrc1* single-mutant cells together with the substantial SAC activation in achiasmate *mrc1 rec12* double-mutant cells, sister kinetochores probably face the same side in *mrc1* mutants. However, the frequent bipolar attachment of sister chromatids seen in *mrc1 rec12* mutant cells conversely implies that the kinetochores face opposite sides. This contradiction may be explained by the flexibility of the kinetochore arrangement (Figure S5A, Text S1). It is possible that in the *mrc1* mutant cells, although sister kinetochores are initially arranged side by side, the kinetochores end up facing opposite sides when they are pulled from opposite directions, leading to the subsequent efficient bipolar attachment of sister centromeres.

On the other hand, in *moa1* mutant cells, sister kinetochores perhaps face opposite sides to attach to both poles efficiently (Figure S5A, Text S1), as proposed previously [34]. Although kinetochore arrangement was previously proposed to be flexible in *moa1* mutant cells [34], we speculate that the arrangement is conversely inflexible because of strong centromere cohesion, considering increased centromere accumulation of cohesin [34], infrequent sister centromere dissociation (Figure 6B), and a narrower dissociation distance (Figure S6). Bipolar attachment was not eliminated in *moa1* single-mutant cells, perhaps because bipolar attachment is easily re-established due to the back-to-back kinetochore arrangement. An alternative possibility is that *moa1* mutant cells are defective in destabilizing the kinetochore–microtubule interaction and fail to eliminate improper attachments efficiently.

Common mechanisms for chromosome segregation between mitosis and meiosis

Our findings have three important implications for understanding the mitotic chromosome segregation mechanism. First, the frequent bipolar attachment of sister chromatids seen in achiasmate cells indicates that kinetochore arrangement alone cannot prevent improper attachments and suggests that bipolar (merotelic) attachment of a single chromatid also occurs when sister chromatid cohesion is defective. Indeed, Courtheoux et al.

recently reported that merotelic attachments occur during mitotic anaphase in *rad21* fission yeast mutants defective in sister chromatid cohesion [53]. Furthermore, a lagging chromatid was frequently observed during anaphase II in *sgo1* mutant of fission yeast, in which sister chromatids undergo precautious dissociation before anaphase II [17]. These observations may alter the interpretation of phenotypes associated with monopolin and heterochromatin mutants of fission yeast, which were proposed to be defective in the arrangement of microtubule-binding sites of kinetochores because these mutants frequently exhibited merotelic attachments during mitotic anaphase [54,55]. However, defective sister centromere cohesion in the monopolin and heterochromatin mutants may have caused the merotelic attachments [56–58].

Second, the fact that sister chromatids, despite their bipolar attachment, move to the same pole in chiasmate cells indicates that monopolar attachment of sister chromatids is not a prerequisite for their proper segregation. This feature is probably common during mitotic chromosome segregation because the proper segregation of a single chromatid that is attached to both poles has also been observed in higher eukaryotes during mitosis [59]. Therefore, generation of bias in the segregation forces is probably a general mechanism that ensures correct chromosome segregation.

Finally, the chromosome oscillation-dependent model for the elimination of improper attachments may also account for the establishment of proper attachments during mitosis (Figure S5B). During mitosis, chromosomes oscillate during the establishment of their spindle attachment (Figure S4A) [60,61], and merotelic attachment occurs in higher eukaryotes [62]. Furthermore, in fission yeast, the physical linkage between two kinetochores induces their bipolar attachment during mitosis [63]. These facts suggest that the oscillation of cohesin-linked sister chromatids destabilizes improper attachments and contributes to the selection of proper attachments during mitosis.

In summary, we have shown that chiasmata are essential for proper spindle attachment and segregation of sister chromatids during meiosis I. Based on our results, we propose that chiasmata play a pivotal role in the selection of proper attachments and establish a backup mechanism that promotes the appropriate segregation of chromosomes when improper attachments remain during anaphase I. Furthermore, we propose a model to explain how chromosome association contributes to correct spindle attachment of the chromosomes not only in meiosis but also in mitosis. Our findings increase understanding of the general mechanisms of chromosome segregation and contribute to knowledge about the mechanisms that underlie the chromosome mis-segregation associated with birth defects and/or tumorigenesis in humans.

Materials and Methods

Yeast strains and media

Table S3 lists the yeast strains used in this study, and strains used in figures are described in Text S1. Media used in this study have been described by Moreno et al. [64].

Analysis of chromosome segregation during meiosis I in diploid cells

Yeast strains were grown on solid YES medium at 30°C. For the segregation analyses of homologous chromosomes, two types of cells, both of which contained GFP-labeled centromeres (*cen2* or *lys1*), were crossed on solid ME medium. For sister chromatid segregation analyses, cells containing GFP-labeled centromeres were crossed with cells lacking GFP-labeled centromeres. The resulting diploid cells were then induced to enter meiosis by incubation at 25°C for 16–18 h. Nuclear DNA in meiotic zygotes was stained with the DNA-specific dye, Hoechst 33342, as described [65]. GFP signal was examined in zygotes containing two round DNA masses that underwent meiosis I. Zygotes containing two DNA masses with a tear-drop shape and pointed ends facing each other were excluded because they were in the karyogamy stage.

Analysis of chromosome segregation during meiosis I in haploid cells

Haploid yeast cells were forced to enter meiosis by Pat1 inactivation following activation of the mating pheromone signaling pathway, as previously described [33]. Haploid *pat1* temperature-sensitive mutant cells bearing the *c*-type *mat* gene of the opposite mating type, which is required for activation of the mating pheromone signaling pathway, were grown in YES-rich medium to a density of $3–5\times10^6$ cells/ml at 25°C. The cells were suspended in an equal volume of EMM2 medium lacking a source of nitrogen (EMM2-N) and incubated at 25°C for 14–16 h to synchronize the cells in G1 phase and activate the mating pheromone signaling pathway. The cells were resuspended in fresh EMM2-N medium and induced to enter meiosis by further incubation at 34°C. Meiotic progression was monitored by analysis of chromosomal DNA morphology at 1-h time intervals. Sister chromatid segregation was analyzed in cells containing two DNA masses that underwent meiosis I.

Live cell analysis of chromosome and spindle pole dynamics

The chromosome locus and spindle poles were visualized using the lacI/lacO recognition system and the GFP-tagged spindle pole component Sid4, respectively, as described previously [42]. Cells were grown on solid YES medium at 30°C and induced to undergo meiosis by incubation on solid ME medium at 25°C for 16–18 h. The cells were observed to determine the dynamics of the GFP-labeled spindle pole or chromosome locus at 25°C using a DeltaVision microscope system (Applied Precision Inc.) equipped with a 60X/1.42 numerical aperture Plan Apo oil-immersion objective lens (Olympus), as described previously [65]. The behavior of the GFP-labeled chromosome locus was observed every 1 min or 10 s. A set of images from six focal planes with 0.5-μm intervals or ten focal planes with 0.3-μm intervals was taken at each time point for 1-min or 10-s time-lapse analysis, respectively. Behavior of the GFP-tagged Cnp1 was observed in a manner similar to the 10-s time-lapse analysis of the GFP-labeled chromosome locus, except that a 100X/1.4 numerical aperture Plan Apo oil-immersion objective lens (Olympus) was used. All measurements were conducted in three dimensions.

Supporting Information

Figure S1 Chromosome dynamics at meiosis I in haploid cells. Haploid cells containing transcriptionally active mating type genes of the P and M types were induced to enter meiosis by nitrogen starvation [33]; chromosomes and the spindle were respectively visualized by DNA specific dye, Hoechst 33342, and GFP-tagged α2-tubulin, and their behavior was monitored at meiosis I, as previously described [33]. Magenta and green show chromosomes and the spindle, respectively. Numbers indicate time in minutes. Arrowheads indicate three chromosomes, and arrows indicate lagging chromosomes during anaphase I. 3 out of 5 examined cells showed lagging chromosomes.
Found at: doi:10.1371/journal.pgen.1001329.s001 (0.95 MB TIF)

Figure S2 Anaphase initiation timing and spindle dynamics in various types of cells. (A) Timing of anaphase I onset examined by

phase II duration of spindle elongation. Phase II duration of spindle elongation was examined, as previously reported [42]. The illustration shows typical elongation of the meiosis-I spindle over time and three phases in spindle elongation. Phase II and phase III presumably correspond to prometa/meta-phase and anaphase, respectively. Error bars show standard deviation. Values of spindle duration in Wt, *mad2*, *rec12*, and *mad2 rec12* cells were adopted from our previous manuscript [42]. +: no mutations otherwise depicted. The number of spindles examined is shown in parentheses. Asterisks show durations that are statistically different from the duration of wild type and their associated p values, as determined by t-tests. * $p<0.05$; ** $p<0.005$; *** $p<1\times10^{-5}$. (B) Spindle dynamics. Photos show spindle dynamics in *mrc1 rec12* and *moa1 rec12* mutants. Each graph shows changes in the length of 6 spindles.
Found at: doi:10.1371/journal.pgen.1001329.s002 (2.36 MB TIF)

Figure S3 Spore viability and chromosome segregation in *mrc1* mutant. (A) Number of spores formed in wild-type and *mrc1* asci. Graph shows average percentages obtained from two independent experiments. More than 250 asci were examined for each strain in each experiment. (B) Average spore viability of wild type and *mrc1* mutant. Four spores in wild-type and *mrc1* asci were dissected and examined for their viability by colony formation. Average spore viabilities of wild type and *mrc1* mutant were obtained respectively from 4 and 7 independent experiments. At least 10 asci were dissected in each experiment. (C) Meiotic segregation of both homologous chromosomes and sister chromatids. Cells containing GFP-visualized *cen2* of both homologous chromosomes were induced to meiosis, and chromosome segregation was examined in four nuclear cells that completed two divisions. Bars show percentages of cells containing four nuclei, each of which contains a single GFP signal. 76 and 85 cells were examined for wild type and *mrc1* mutant, respectively. (D) Sister chromatid segregation at meiosis II. Segregation of sister chromatids at meiosis II was examined by segregation patterns of GFP-visualized *cen2* of one of the homologous chromosomes in four nuclear cells, and bars show average percentages of sister chromatid disjunction (black) and non-disjunction (white) at meiosis II. Cells which segregated sister chromatids equationally at meiosis I were excluded. The percentages in wild-type, *mrc1*, and *sgo1* mutant cells were obtained from 2, 4, and 3 independent experiments, respectively, and more than 40 zygotic cells were examined in each experiment. Error bars in (B) and (D) indicate standard deviation.
Found at: doi:10.1371/journal.pgen.1001329.s003 (0.35 MB TIF)

Figure S4 Centromere dynamics during the mitotic pre-anaphase stage in diploid wild-type cells. (A) Pre-anaphase dynamics of the spindle pole and centromere (*cen2*). Photos were taken every 10 s and are shown in order from left to right. Horizontal bar: 50 s. bar: Vertical 2 μm. Graph shows changes in distance between the SPB and the centromere (red, SPB#1-cen#1) and between the two SPBs (black, SPB#1-SPB#2). (B) Average dissociation frequencies and duration of sister centromeres. 8 pairs of sister centromeres were examined. At least 10 consecutive time points were examined for each analysis. (C) Observation frequencies of centromeres at distinct positions in the spindle during the pre-anaphase stage. The positions of centromeres are shown based on their relative distance from the spindle center (d), as determined in Figure 4B. The number of examined positions is shown in parenthesis.
Found at: doi:10.1371/journal.pgen.1001329.s004 (0.46 MB TIF)

Figure S5 Models for spindle attachment in various mutants and chromosome oscillation-dependent elimination of improper attachments. (A) Sister kinetochore arrangement and changes in spindle attachment of the kinetochores after their attachment to opposite poles (Bipolar attachment). Tension generation: generation of tension (white arrows) on sister kinetochores by microtubules. Selection: elimination of kinetochore-interacting microtubules. Amplification: increase of kinetochore-interacting microtubules. (B) Chromosome oscillation model for selection of proper attachments. Bipolar attachments of sister centromeres (Meiosis I, centromeres on left sister chromatids) or a single centromere (Mitosis, a left centromere) occasionally occurs, and tension (white arrows) is generated at the centromeres (Improper attachment). Movement of chiasma- or cohesin-linked chromosomes eliminates tension generated at the sites of improper attachments at meiosis I or in mitosis, respectively (Loss of tension), because the leading chromosome(s) exerts pulling forces (small gray arrows) on the following chromosome(s) at the chromosome linkage sites. Elimination of tension leads to detachment of improperly interacting microtubules (Detachment). Large gray arrows in (A) and (B) indicate chromosome movement.
Found at: doi:10.1371/journal.pgen.1001329.s005 (0.48 MB TIF)

Figure S6 Average distance between dissociated sister centromeres. Asterisks show distances that are statistically different from the distance of wild type (+, *rec12*$^{+}$) and their associated p values, as determined by t-tests. * $p<0.05$; ** $p<2\times10^{-6}$; *** $p<1\times10^{-23}$. +: no mutations otherwise depicted. ND: not determined. Error bars indicate standard deviation. The number of examined distances is the following. Wt: 181; *rec12*: 263; *mrc1*: 127; *mrc1 rec12*: 219; *moa1*: 197; *moa1 rec12*: 125; *rec8*: 197. In *moa1* mutants, the distance between dissociated sister centromeres was significantly shorter than in wild-type cells. In contrast, this distance was significantly longer in *rec8* mutant cells (*rec8Δ*).
Found at: doi:10.1371/journal.pgen.1001329.s006 (0.12 MB TIF)

Table S1 Parameters of centromere movements during the pre-anaphase stage at meiosis I.
Found at: doi:doi:10.1371/journal.pgen.1001329.s007 (0.05 MB DOC)

Table S2 Direction of centromere movements during the pre-anaphase stage at meiosis I.
Found at: doi:10.1371/journal.pgen.1001329.s008 (0.05 MB DOC)

Table S3 Strain list [67–74].
Found at: doi:10.1371/journal.pgen.1001329.s009 (0.10 MB PDF)

Text S1 Supplementary text [66].
Found at: doi:10.1371/journal.pgen.1001329.s010 (0.08 MB DOC)

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Author Contributions

Conceived and designed the experiments: A Yamamoto, Y Hirose, R Suzuki. Performed the experiments: A Yamamoto, Y Hirose, R Suzuki, T Ohba, Y Hinohara, H Matsuhara, M Yoshida, Y Itabashi. Analyzed the data: A Yamamoto, Y Hirose, R Suzuki, T Ohba, Y Hinohara, H Matsuhara, M Yoshida, Y Itabashi. Contributed reagents/materials/analysis tools: H Murakami. Wrote the paper: A Yamamoto.

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