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Spatial Regulation of Kinetochore Microtubule Attachments by Destabilization at Spindle Poles in Meiosis I

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SUMMARY

To ensure accurate chromosome segregation in cell division, erroneous kinetochore-microtubule (MT) attachments are recognized and destabilized [1]. Improper attachments typically lack tension between kinetochores and are positioned off-center on the spindle. Low tension is a widely accepted mechanism for recognizing errors [2], but whether chromosome position regulates MT attachments has been difficult to test. We exploited a meiotic system in which kinetochores attached to opposite spindle poles differ in their interactions with MTs and therefore position and tension can be uncoupled. In this system, homologous chromosomes are positioned off-center on the spindle in oocytes in meiosis I, while under normal tension, as a result of crossing mouse strains with different centromere strengths, manifested by unequal kinetochore protein levels [3]. We show that proximity to spindle poles destabilizes kinetochore-MTs and that stable attachments are restored by inhibition of Aurora A kinase at spindle poles. During the correction of attachment errors, kinetochore-MTs detach near spindle poles to allow formation of correct attachments. We propose that chromosome position on the spindle provides spatial cues for the fidelity of cell division.

RESULTS AND DISCUSSION

Proper chromosome segregation during eukaryotic cell division requires that kinetochores attach to opposite spindle poles (biorientation) so that sister chromatids (mitosis/meiosis II) or homologous chromosomes (meiosis I, MI) are pulled in opposite directions in anaphase. Incorrect attachments are selectively destabilized to allow new attachments to form (reorientation). During this error-correction process, it is widely accepted that kinetochore-microtubule (MT) interactions are regulated by tension, due to MTs pulling kinetochores toward opposite spindle poles [2]. Kinetochore substrates of Aurora B kinase (AURKB), which localizes to the inner centromere, are phosphorylated when tension is low to destabilize incorrect attachments [4]. This process has been studied in mitotic cells in the context of syntelic attachment errors, in which sister kinetochores are attached to the same spindle pole. AURKB activity leads to depolymerization of syntelic kinetochore-MTs, but attachments are maintained as chromosomes are pulled toward the pole [5]. From the pole, chromosomes then congress and ultimately achieve biorientation by capturing MTs from the opposite site of the spindle [6]. Because low tension does not directly lead to MT release from kinetochores, it is unclear how erroneous MTs are detached to allow reorientation. The observation that syntelic chromosomes approach the spindle pole as part of the error correction process suggests that chromosome position on the spindle may contribute to release of kinetochore-MTs.

Uncoupling mechanisms that depend on chromosome position versus tension has been challenging because chromosomes near spindle poles are also likely to be incorrectly attached and lack tension. Furthermore, most chromosomes align quickly in the center of the spindle in mitosis, limiting opportunities to examine spatial regulation. To overcome these problems, we examined mouse oocytes in MI with asymmetric homologous chromosomes, which are typically positioned off-center on the spindle while correctly oriented toward opposite spindle poles. We used oocytes with a single Robertsonian (Rb) chromosome, which is a metacentric chromosome created by fusion of two telocentric chromosomes (6 and 16) at the centromeres. We crossed a standard laboratory strain with all telocentric chromosomes (CF-1) to a strain homozygous for the Rb(6.16) fusion. In MI oocytes from the offspring from the Rb(6.16) × CF-1 cross, the Rb fusion is in the heterozygous state and pairs with the two homologous telocentric chromosomes, creating an asymmetric trivalent (Figure 1A). Within the trivalent, we previously showed that centromeres of the telocentrics are stronger than the fusion centromere, as indicated by higher levels of kinetochore proteins [3]. These differences in centromere strength lead to unbalanced MT interactions that position the trivalent closer to one spindle pole (Figures 1B–1D). In addition to the single trivalent, these oocytes also contain symmetric bivalents that align normally at the spindle mid-zone. The trivalent was stretched similarly to bivalents, based on distances measured between centromeres of homologous chromosomes, indicating that the trivalent is under normal tension (Figure 1E). In comparison, inter-centromere distance was reduced in cells treated with a kinesin-5 inhibitor, which generates monopolar spindles that cannot exert tension. Tension and position are therefore

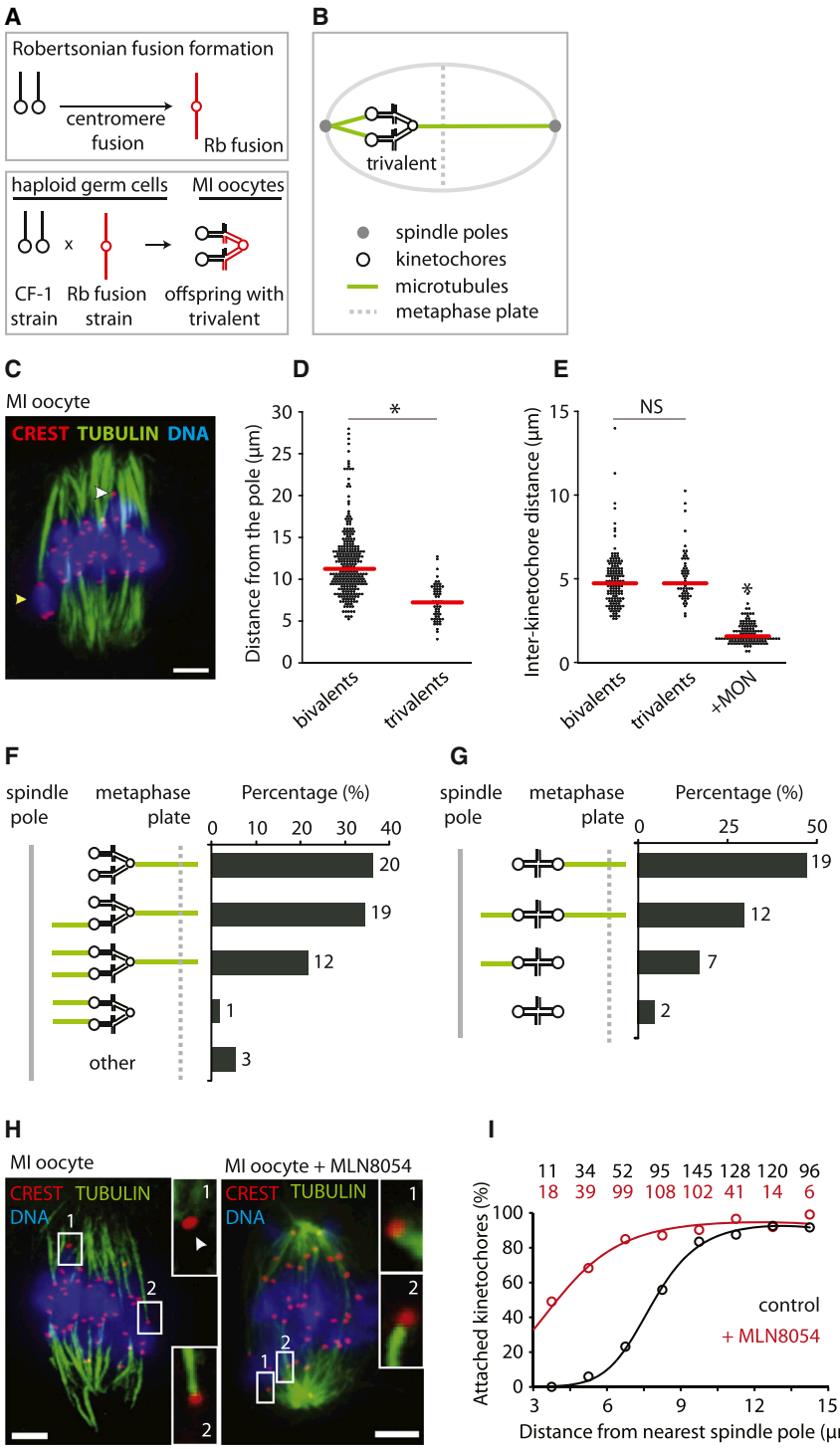

Figure 1. Proximity to Spindle Poles Destabilizes Kinetochore-MTs, Dependent on AURKA Activity

(A) An Rb fusion metacentric pairs with the two homologous telocentric chromosomes in MI to form a trivalent.

(B) The trivalent is typically positioned off-center with the two telocentrics near the spindle pole.

(C–I) Rb(6.16) × CF-1 oocytes were fixed at metaphase I and analyzed for cold-stable MTs. Images (C and H) are maximal intensity z projections showing centromeres (CREST), tubulin, and DNA; arrowheads indicate unattached kinetochores of a trivalent (yellow) and a bivalent (white) positioned near the spindle poles, and insets are optical sections showing individual kinetochores. Scale bars represent 5 μm. The distance between kinetochores and the nearest spindle pole (D) was measured for telocentrics in trivalents (n = 28) and bivalents (n = 280; half of the data points are displayed). Inter-kinetochore distance (E) was measured between homologous centromeres of the bivalents, between centromeres of telocentrics and Rb metacentrics in the trivalents, and between homologous centromeres of bivalents in monastrol-treated cells (n = 220; half of the data points are displayed). Schematics show the MT attachment configurations and frequency for trivalents (F) and bivalents (G) positioned off-center on the spindle. Numbers indicate chromosomes counted in each category, from multiple independent datasets. For controls (H) or oocytes treated with 5 μM of the AURKA inhibitor MLN8054 for 1 hr before fixation (I), kinetochores from both bivalents and trivalents were binned in 1.5-μm intervals based on distance from the nearest spindle pole, and the fraction of attached kinetochores was calculated in each bin. Lines show the logistic regression curves, based on parameters in Table S1. Numbers above each data point represent total numbers of kinetochores in each bin.

*p < 0.001; NS, not significant. See also Figure S1 and Table S1.

uncoupled for the trivalents, allowing us to test effects of position while under normal tension.

To visualize kinetochore-MTs in the trivalent, we used a cold-stable MT assay [7], as kinetochore-MTs are preferentially stabilized at 4°C while other MTs depolymerize. We found that kinetochores of the telocentric chromosomes, positioned closer to the spindle poles, frequently lacked cold-stable attachments (59/104, 57%), whereas the homologous fusion kinetochore, positioned farther from the pole, was rarely unattached (1/52, 2%) (Figure 1F). We also occasionally observed normal bivalents positioned off-center, most likely due to high-amplitude oscillations [8]. These bivalents showed similar behavior as the trivalents: kinetochores near the spindle pole generally lacked cold-stable MT attachments (21/40, 52.5%), whereas the kinetochores farther from the pole were less frequently unattached (9/40, 22.5%) (Figure 1G). Our finding that tension can be exerted without cold-stable attachments is consistent with previous observations. In mouse oocytes, inter-centromere distance is maximal even before cold-stable kinetochore-MTs are established [7–9]. Furthermore, increasing Aurora B activity at mitotic kinetochores leads to loss of cold-stable attachments without

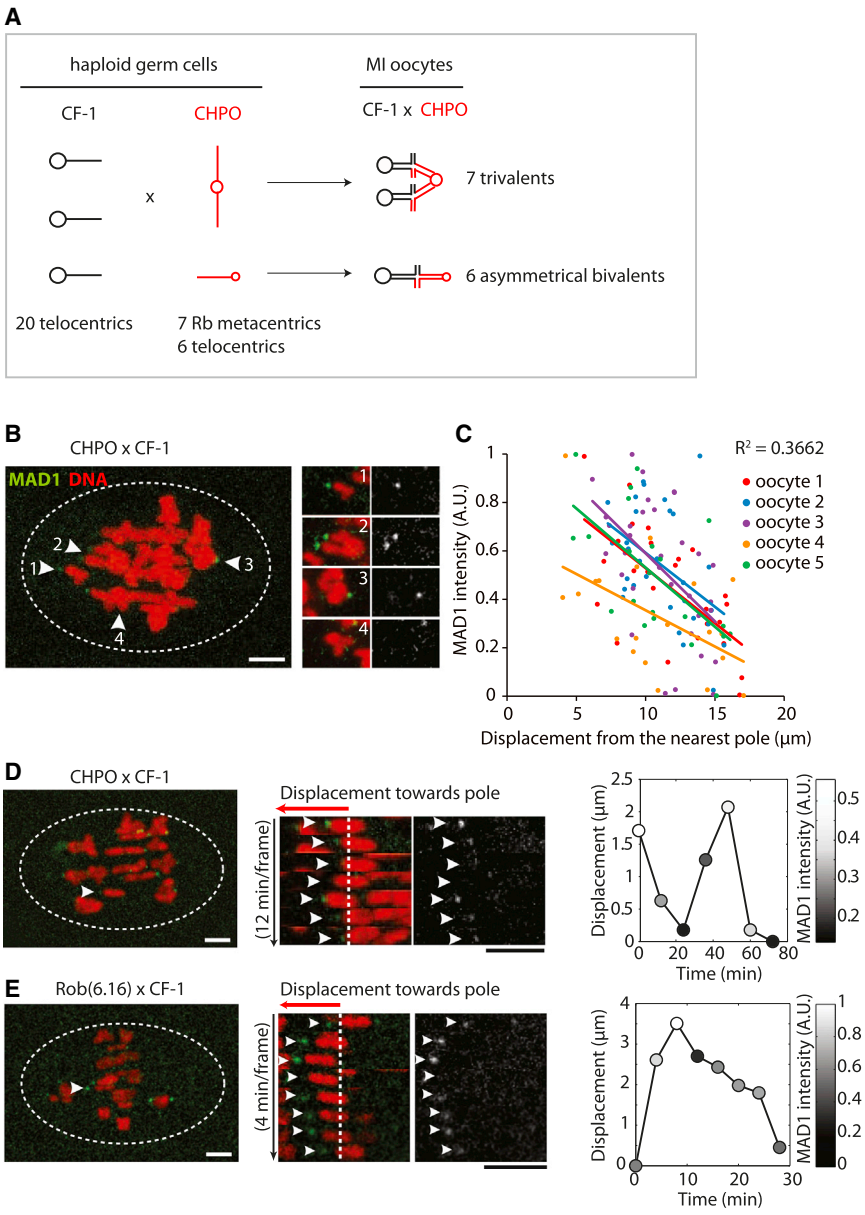

Figure 2. Kinetochores Accumulate MAD1 as They Approach Spindle Poles

(A) Chromosome composition in CF-1 and CHPO and in CHPO × CF-1 MI oocytes.

(B–E) CHPO × CF-1 (B–D) or Rb(6.16) × CF-1 (E) oocytes expressing MAD1-2EGFP and histone H2B-mCherry were imaged live. An optical section (B) shows chromosomes near spindle poles (arrowheads and insets 1–3) and at the metaphase plate (inset 4). Kinetochore MAD1-2EGFP intensity is plotted versus distance from the nearest spindle pole (C); colors indicate kinetochores ($n > 15$) from five different oocytes. R^2, cumulative correlation coefficient for all oocytes for a linear regression model; $p < 0.0001$. MAD1-2EGFP intensity was tracked on kinetochores of oscillating bivalents (D and E). Images are optical sections; arrowheads indicate kinetochores tracked in the kymographs, and dashed ovals indicate spindle outlines. The graphs show MAD1-2EGFP intensity and displacement toward the pole over time course. Scale bars represent 5 μm.

See also Figures S2 and S3.

loss of tension [10]. Overall, our results suggest that proximity to spindle poles destabilizes kinetochore-MTs for both trivalent and normal bivalent chromosome configurations.

Aurora A kinase (AURKA) belongs to the same family as AURKB, sharing 71% sequence identity in the kinase domain, and phosphorylates many of the same substrates [11–14]. AURKA localizes to spindle poles, which suggests that its activity may destabilize kinetochore-MTs near the poles. To test this model, we partially inhibited AURKA activity with MLN8054, a small-molecule inhibitor that is 150-fold more selective for AURKA versus AURKB and is relatively ineffective toward most other kinases [15]. Because full inhibition of AURKA severely disrupts the spindle, we used a concentration (5 μM) that reduces phosphorylation of T288, crucial in kinase auto-activation [16], by ~40%, with a moderate effect on spindle size (Figures S1A and S1B). Treatment with MLN8054 did not affect AURKB activity, as measured by staining with a phospho-specific antibody against the C-terminal TSS motif of INCENP [17] (Figures S1C and S1D), which is a useful marker for AURKB activity because it is phosphorylated by AURKB as part of the mechanism of kinase activation [18–20]. We found that kinetochore-MTs were frequently stabilized near spindle poles after partial AURKA inhibition (Figure 1H).

To quantify the relationship between kinetochore-MT attachments and distance from the spindle poles, we scored cold-stable MTs for kinetochores near the poles as well as randomly chosen kinetochores at the metaphase plate, and measured their distance from the nearest pole. We fit a quadratic logistic regression model to the data (Figure 1I and Table S1). The regression curve for AURKA inhibition was significantly shifted toward shorter distances, indicating that the probability of forming stable MT attachments near the poles was significantly higher when AURKA was partially inhibited. In contrast, reduction of spindle size to comparable levels by partial inhibition of kinesin-5 did not affect the relationship between attachment stability and distance from spindle poles (Figures S1E–S1G and Table S1). These results indicate that AURKA activity destabilizes kinetochore-MTs near spindle poles.

To establish a live imaging assay for kinetochore-MT attachments, we monitored levels of the checkpoint protein MAD1, which is recruited to kinetochores lacking stable MTs and is removed when stable attachments form [21]. We injected oocytes with cRNAs coding for MAD1-2EGFP, with two EGFPs at the C terminus, and histone H2B-mCherry to label chromosomes. For these experiments, we used Rb(6.16) × CF-1 oocytes with a single trivalent (Figure 1) and another system

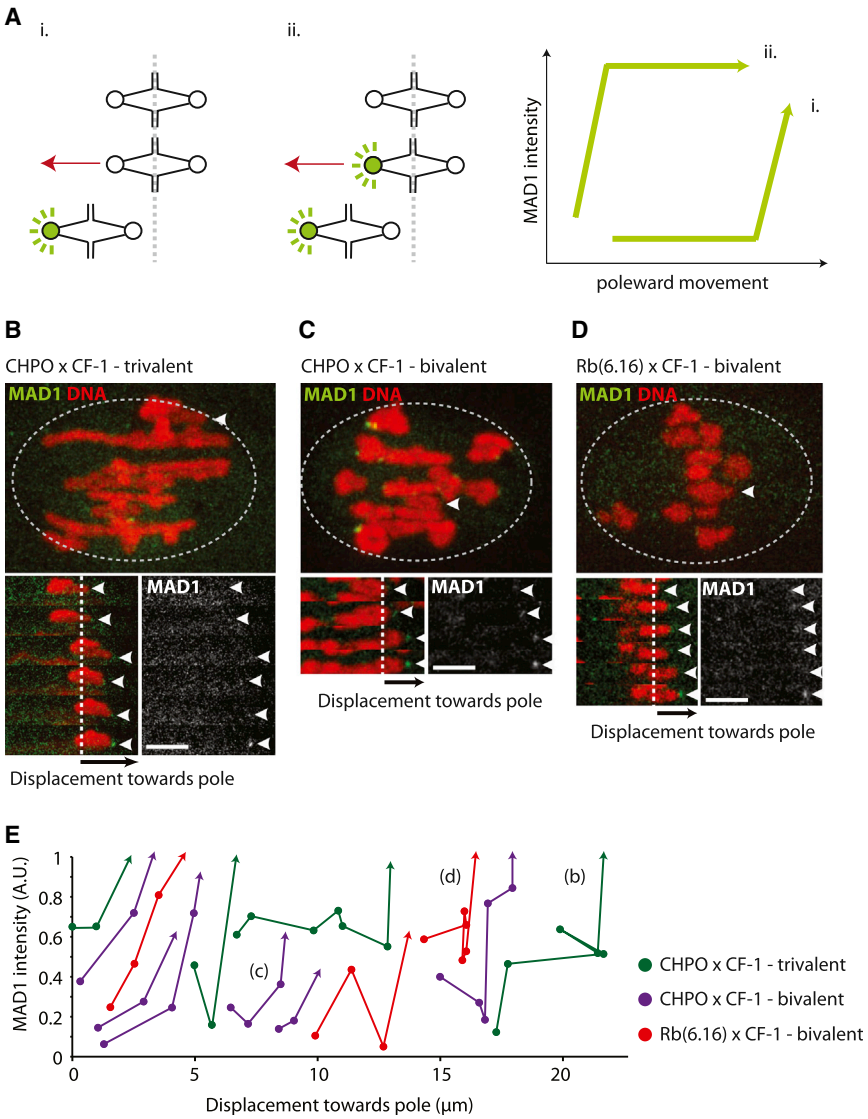

Figure 3. Kinetochore Poleward Movement Precedes MAD1 Accumulation

(A) Schematics showing two models: chromosome poleward movement precedes (i) or follows (ii) MAD1-2EGFP accumulation and how they can be distinguished graphically.

(B–E) CHPO × CF-1 or Rb(6.16) × CF-1 oocytes expressing MAD1-2EGFP and histone H2B-mCherry were imaged live. Images (B–D) are optical sections; arrowheads indicate kinetochores tracked in the kymographs, and dashed ovals indicate spindle outlines. MAD1-2EGFP intensity is plotted versus displacement toward the pole (E) for 13 individual kinetochores. Data points are sequential time points, with the last time point indicated by the arrowhead. Individual traces are horizontally offset by an arbitrary distance for visual clarity. Scale bars represent 5 μm.

(CHPO × CF-1 oocytes) with more chromosomes positioned close to spindle poles. CHPO is a strain homozygous for seven Rb fusions [22, 23], so CHPO × CF-1 oocytes contain seven trivalents and six bivalents. We previously showed that CHPO centromeres are weaker overall than CF-1 centromeres [3]. CHPO × CF-1 bivalents and trivalents are therefore asymmetric, because weak (CHPO) centromeres are paired with strong (CF-1) centromeres (Figure 2A), and are frequently positioned near spindle poles due to unbalanced MT interactions. Within CHPO × CF-1 bivalents and trivalents, we found that kinetochores near the spindle poles frequently have higher levels of MAD1-2EGFP than kinetochores of the homologous chromosomes farther from the pole (Figure 2B). Furthermore, MAD1-2EGFP intensity is negatively correlated with kinetochore distance from the spindle pole (Figure 2C). AURKA inhibition, either with MLN8054 or by overexpression of a kinase-dead mutant (AURKA-KD) [24], led to loss of MAD1-2EGFP from kinetochores close to spindle poles (Figure S2), consistent with the increase in cold-stable MTs (Figures 1H and 1I). Conversely, overexpressing wild-type AURKA led to reduced cold-stable kinetochore-MTs at all kinetochores and almost complete loss near the spindle poles (Figures S3C–S3F). Consistent with this observation, EGFP-AURKA localized not only to spindle poles, but also weakly to kinetochores (Figure S3A).

We observed several examples of bivalents with high amplitude oscillations that approached the poles, both in CHPO × CF-1 oocytes and Rb(6.16) × CF-1 oocytes. Within each bivalent, the kinetochore closer to the pole accumulated MAD1-2EGFP as it moved toward the pole and lost MAD1-2EGFP as it moved away (Figures 2D and 2E). Overall, our analyses of MAD1-2EGFP recruitment in live cells are consistent with our findings that kinetochore-MT stability correlates with distance from the spindle poles in fixed oocytes (Figure 1I).

If kinetochore-MTs are destabilized due to proximity to spindle poles, we predict that chromosome movement would precede MAD1-2EGFP accumulation (Figure 3A, i). Alternatively, chromosomes could move toward the poles because attachments are destabilized on one side, in which case movement would follow MAD1-2EGFP accumulation (Figure 3A, ii). To distinguish between these possibilities, we analyzed bivalents and trivalents that were moving toward the spindle pole with detectable kinetochore MAD1-2EGFP accumulation in CHPO × CF-1 or Rb(6.16) × CF-1 oocytes. In the majority of instances (11/13), >50% of the total displacement toward the pole occurred before 50% of the total change in kinetochore MAD1-2EGFP intensity (Figures 3B–3E). Otherwise, MAD1-2EGFP accumulation occurred synchronously with, but not before, displacement toward the spindle pole. Overall, these results demonstrate that chromosome poleward movement leads to kinetochore-MT destabilization.

During the correction of syntelic attachment errors, chromosomes move toward the spindle pole while maintaining

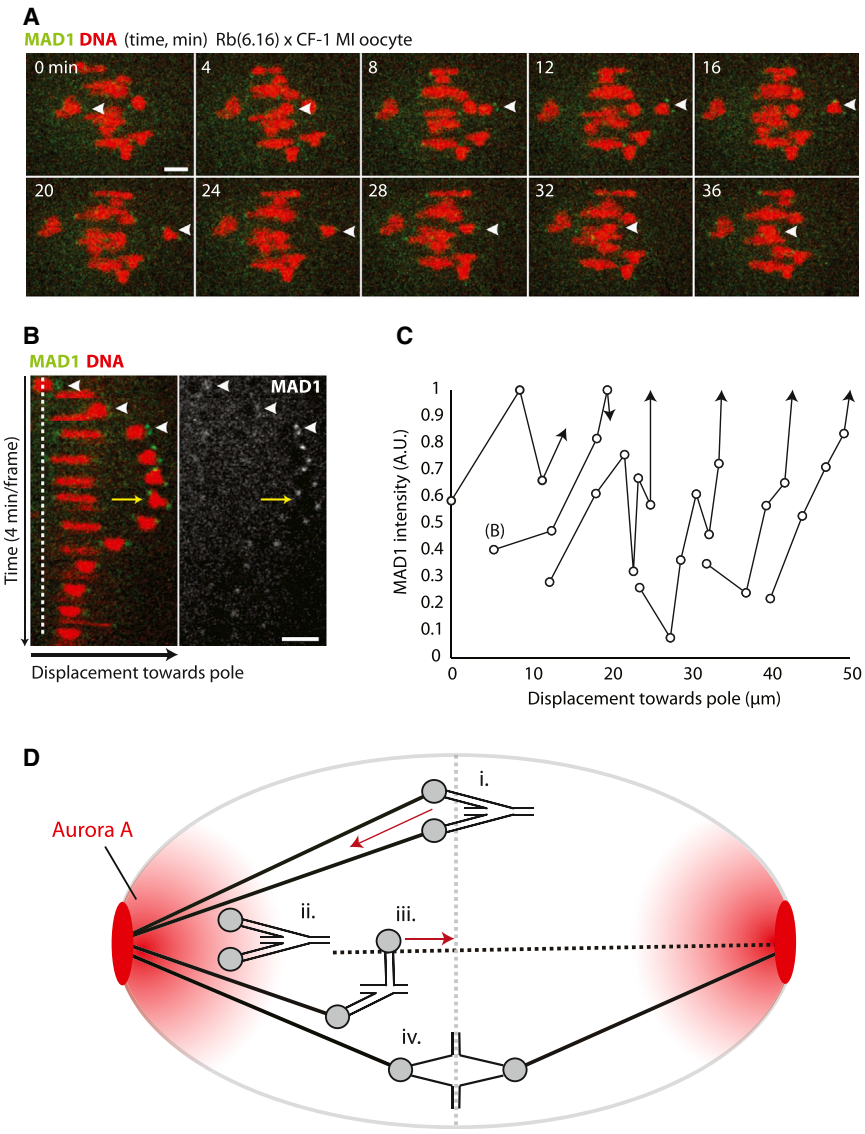

Figure 4. MAD1 Accumulates on Kinetochores of Syntelic Chromosomes as They Approach the Spindle Pole during Error Correction

(A–C) Oocytes expressing MAD1-2EGFP and histone H2B-mCherry were imaged live during correction of syntelic attachment errors. Images (A) are optical sections from a time lapse; timestamps show hr:min, and arrowheads indicate the bivalent tracked in the kymograph (B). Yellow arrows indicate reorientation of the kinetochores to face opposite poles. MAD1-2EGFP intensity was summed over both kinetochores in the syntelic and plotted versus displacement toward the pole (C) for individual bivalents, from Rb(6.16) × CF-1 (n = 4) or SPRET × C57BL/6 (n = 2) oocytes. For clarity, only poleward movements are plotted. Data points are sequential time points, with the last time point being indicated by the arrowhead. Individual traces are horizontally offset by an arbitrary distance for visual clarity. Scale bars represent 2.5 μm.

(D) Model for correction of syntelic attachment errors: low tension leads to kinetochore-MT disassembly and poleward movement (i), MTs detach from kinetochores near the spindle poles due to AURKA activity (ii), and chromosomes reorient by congressing toward the metaphase plate and capturing MTs from the opposite spindle pole (iii and iv).

See also Figure S4.

kinetochore-MT attachments [5]. Our results suggest that these MTs would release as chromosome approach the pole. To test this prediction, we identified syntelically attached bivalents and analyzed kinetochore MAD1-2EGFP as they moved toward the spindle pole. For these experiments we used Rb(6.16) × CF-1 oocytes, which have normal, symmetric bivalents that ultimately align at the metaphase plate. Initially, we observed low kinetochore MAD1-2EGFP for syntelics moving from the center of the spindle toward the pole, indicating that lack of tension was sufficient to trigger MT disassembly, but not detachment (Figures 4A–4C), which would drive poleward movement, consistent with previous observations in mitotic cells [5]. Kinetochore MAD1-2EGFP levels increased after the syntelics were drawn toward the spindle pole (within 2–3 μm from the pole), indicating that MT attachments were released at the spindle poles. In several cases (three out of five), we observed reorientation, as one unattached kinetochore rotated to face the opposite pole, followed by congression to the metaphase plate (Figures 4A and 4B). These results demonstrate that kinetochore-MTs detach near spindle poles during correction of syntelic attachment errors.

Overall, we show that kinetochore-MTs are destabilized near spindle poles in MI and that stable attachments are restored by AURKA inhibition. When kinetochores are positioned near the poles, either due to asymmetric centromere strength or during correction of syntelic errors, we observed increased levels of MAD1-2EGFP. Correctly attached, symmetric bivalents rarely approach close to spindle poles and are therefore not destabilized. Our results support a three-step model for correcting syntelic attachment errors (Figure 4D). Initially, increased phosphorylation of AURKB substrates at kinetochores under low tension leads to kinetochore-MT disassembly, which pulls chromosomes toward the spindle pole [5] (Figure 4D, i). AURKA activity at spindle poles, or on MTs near the poles (Figures S4A and S4B), subsequently detaches the incorrect attachments (Figure 4D, ii). Finally, chromosomes congress to the metaphase plate through lateral interactions mediated by CENP-E and achieve biorientation as they move away from the pole [6] (Figure 4D, iii and iv). Our results provide a missing link in the chromosome error correction process, showing that kinetochore-MTs are released by AURKA kinase activity at spindle poles, to allow reorientation.

We propose that spatial regulation of kinetochore-MTs by AURKA near spindle poles is a complementary mechanism to tension-dependent regulation by AURKB at centromeres [1, 25]. This model is consistent with several previous observations in mitotic cells. First, cutting MTs next to one kinetochore by laser microsurgery leads to accumulation of MAD2 on both sister kinetochores near spindle poles [26]. Second, kinetochores positioned close to spindle poles due to loss of the kinesin CENP-E lack attached MTs [27]. Third, there is a high frequency of chromosome alignment and biorientation defects in chicken DT40 cells lacking AURKA, even in the presence of a bipolar spindle [28]. The relative contributions of AURKA and AURKB to destabilizing attachments most likely depend on chromosome position, with AURKB dominant when kinetochores are positioned far from spindle poles, for example during initial stages of syntelic error correction, and cumulative effects of both Aurora kinases contributing to MT release near spindle poles.

At anaphase onset, kinetochore-MTs must be stabilized to support chromosome segregation and prevent re-activation of the spindle checkpoint [29, 30]. To prevent destabilization in response to loss of tension, Aurora B redistributes from centromeres to the spindle mid-zone in anaphase. In addition, Aurora A is degraded at anaphase onset, in both mitotic cells [31] and oocytes (Figure S4C–S4E), which would prevent destabilization as kinetochores approach spindle poles. Therefore, both mechanisms are constrained in anaphase, when maintaining attachments takes priority over error correction. Together, these results suggest that complementary spatial and tension-dependent regulation are a conserved mechanism in meiotic and mitotic cell divisions.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.05.013.

AUTHOR CONTRIBUTIONS

L.C. and K.Y. designed and performed experiments, analyzed data, and wrote the manuscript.

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