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Report

Aurora Kinase Promotes Turnover of Kinetochore Microtubules to Reduce Chromosome Segregation Errors

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Summary

Merotelic kinetochore orientation is a misattachment in which a single kinetochore binds microtubules from both spindle poles rather than just one and can produce anaphase lagging chromosomes, a major source of aneuploidy [1]. Merotelic kinetochore orientation occurs frequently in early mitosis, does not block chromosome alignment at the metaphase plate, and is not detected by the spindle checkpoint [2–5]. However, microtubules to the incorrect pole are usually significantly reduced or eliminated before anaphase [3, 6]. We discovered that the frequency of lagging chromosomes in anaphase is very sensitive to partial inhibition of Aurora kinase activity by ZM447439 at a dose, 3 μM, that has little effect on histone phosphorylation, metaphase chromosome alignment, and cytokinesis in PtK1 cells. Partial Aurora kinase inhibition increased the frequency of merotelic kinetochores in late metaphase, and the fraction of microtubules to the incorrect pole. Measurements of fluorescence dissipation after photoactivation showed that kinetochore-microtubule turnover in prometaphase is substantially suppressed by partial Aurora kinase inhibition. Our results support a preanaphase correction mechanism for merotelic attachments in which correct plus-end attachments are pulled away from high concentrations of Aurora B at the inner centromere, and incorrect merotelic attachments are destabilized by being pulled toward the inner centromere.

Results and Discussion

The Frequency of Anaphase Lagging Chromosomes Is Sensitive to Partial Inhibition of Aurora Kinase

We initially determined that the Aurora kinase inhibitor ZM447439 at a concentration of 3 μM produces a 6-fold increase in anaphase lagging chromosomes over control frequencies in PtK1 cells fixed after 1 hr treatment (Figures 1A and 1B). Notably, a large increase of cells with multiple lagging chromosomes was observed (Figure 1B). Other aspects of mitosis, including chromosome alignment at the metaphase plate (Figure S1 in the Supplemental Data available online) and timing of anaphase onset (data not shown), were relatively unaffected, as determined by phase-contrast time-lapse microscopy. More severe mitotic defects were observed as the dose of ZM447439 increased, and at 20 μM, many different aspects of mitosis were significantly inhibited (Figure S1), as was the Aurora B-dependent phosphorylation of histone H3 at Ser 10 (Figure S2). Three micromolars ZM447439 did not significantly decrease histone H3 phosphorylation as measured by either immunostaining (Figures S2A and S2B) or immunoblotting (Figure S2C) with a phosphohistone H3 antibody.

The increased frequency of anaphase lagging chromosomes at 3 μM ZM447439 suggests that correction of merotelic attachments is very sensitive to partial inhibition of Aurora kinase activity. We used the assay developed by Lampson et al. [7] to test whether 3 μM ZM447439 also affects correction, during prometaphase, of syntelic attachments, in which both sister kinetochores become attached to microtubules from the same pole. Cells were released from Monastrol into media with 3 μM ZM447439 plus MG-132 to prevent anaphase onset. About 50% of the cells did not complete chromosome alignment to the metaphase plate (Figure S3), indicating that the correction of syntelic attachments is also suppressed by 3 μM ZM447439 and that a fully active Aurora kinase is required for correction of kinetochore-microtubule misattachments.

Detachment of kinetochore microtubules from the pole [8] and depolymerization of the kinetochore fiber before reorientation [7] are the suggested mechanisms for correction of syntelic orientation in meiosis and mitosis, respectively. However, recent work on specific centromeric proteins, such as Ipl1-Aurora [9], *m*ammalian *c*entromere-*a*ssociated *k*inesin (MCAK) [10], and its regulator, *i*nner *c*entromere Kin*I* *s*timulator (ICIS) [11], suggests that correction of some misattachments, in particular merotelic attachments, might require microtubule destabilization and detachment at the kinetochore (reviewed in [12]). We have previously observed that merotelic kinetochore orientation can be corrected while the chromosome remains aligned at the metaphase plate and does not depend on complete depolymerization of the kinetochore fiber and poleward movement before reorientation [6]. Therefore, we favor the idea that correction of merotelic orientation occurs by destabilization of microtubule attachments at the kinetochore.

Although ZM447439 has been shown to inhibit both Aurora A and Aurora B in mammalian cells, it also induces the same phenotype resulting from siRNA for Aurora B, not Aurora A [13]. In addition, Aurora B localizes at the centromere in early mitosis in mammalian tissue cells [14], thus representing the best candidate for participating in the correction of kinetochore-microtubule misattachments in early mitosis. On the basis of this, we will discuss our results for correction of merotelic attachments as a consequence of Aurora B inhibition, not Aurora A.

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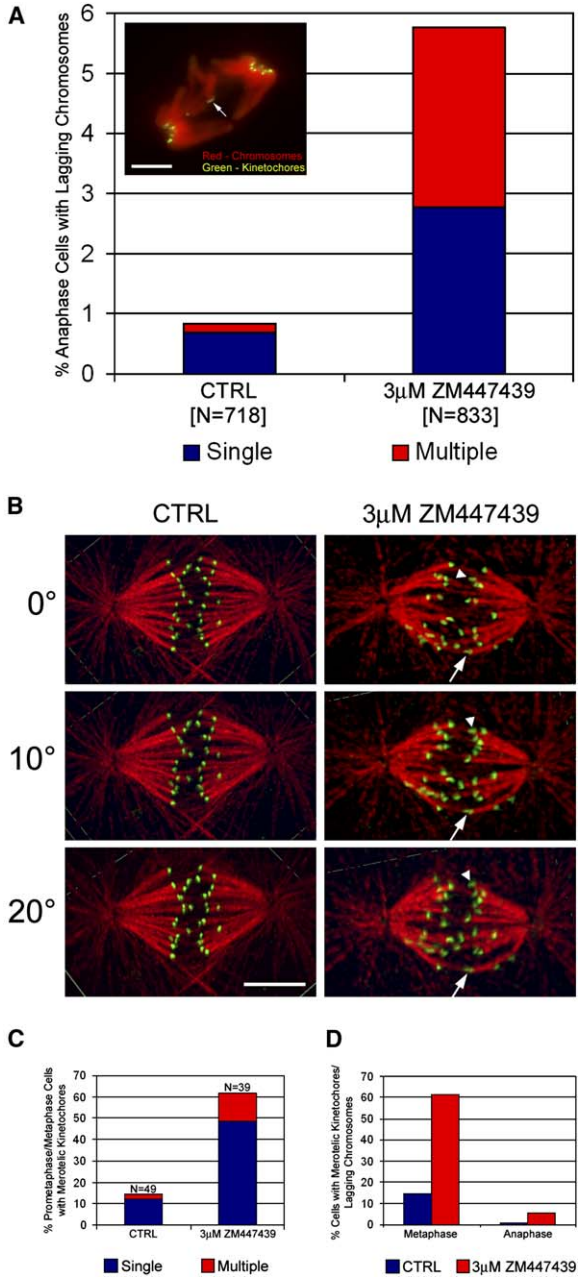

Figure 1. Partial Aurora Kinase Inhibition Induces Anaphase Lagging Chromosomes by Increasing the Number of Merotelic Kinetochores Entering Anaphase

(A) Histogram showing frequencies of anaphase lagging chromosomes in controls (CTRL) and cells treated with 3 μM ZM447439 for 1 hr. The inset at the top left corner shows an anaphase PtK1 cell possessing a lagging chromosome (arrow). The chromosome lags behind at the spindle equator as all the other chromosomes segregate to the spindle poles during anaphase. The scale bar represents 10 μm.

(B) Examples of a normal control metaphase cell (CTRL, left column) and a cell treated with 3 μM ZM447439 for 1 hr (right column). Cells were fixed and immunostained for kinetochores (green) and microtubules (red). The collected images were deconvolved and analyzed with a 3D viewer. The two cells are shown from three different angles as indicated on the left. The treated cell possesses one merotelically oriented kinetochore (arrow). Other kinetochores within the same cell appear connected to microtubule bundles from both spindle poles. However, the 3D image viewer allows discrimination between actual connection (i.e., the kinetochore appears connected to both spindle poles from any angle) and simple overlapping between the kinetochore and a microtubule bundle (compare arrowhead at the 10° angle and arrowheads at 0° and 20°). The scale bar represents 5 μm.

(C) Histogram showing the frequencies of prometaphase and metaphase cells possessing merotelic kinetochores in both untreated cells (CTRL) and cells treated with 3 μM ZM447439.

(D) Histogram comparing frequencies of merotelic kinetochores in late-prometaphase and metaphase cells (Metaphase) to frequencies of anaphase lagging chromosomes (Anaphase) in both untreated cells (CTRL) and cells treated with 3 μM ZM447439.

Partial Inhibition of Aurora Kinase Increases Metaphase Merotelic Kinetochores and Increases the Number of Kinetochore Microtubules to the Incorrect Pole

The movement of merotelically oriented chromosomes away from the equator in anaphase depends on the ratio of kinetochore microtubules to the correct versus incorrect pole [6]. Higher ratios favor poleward movement promoted by anaphase spindle mechanics, whereas a ratio near 1 produces lagging chromosomes at the spindle equator. About 16% of untreated mitotic PtK1 cells enter anaphase with merotelic kinetochores, but only ~1% of these have ratios near 1 and produce anaphase lagging chromosomes [3].

The increase in anaphase lagging chromosomes induced by 3 μM ZM447439 could result either from an increase in merotelically oriented kinetochores in cells entering anaphase or from the inhibition of the prevention mechanism that acts in anaphase and promotes correct segregation of merotelically oriented chromosomes with ratios greater than 1 [6, 15]. To test for the first possibility, we determined the frequencies of merotelically oriented kinetochores in late-prometaphase and metaphase cells. We used 3D image deconvolution of cells immunostained for kinetochores and microtubules (Figure 1B) and found that 61.5% of cells had one or more merotelic kinetochores (>4-fold increase over controls; Figure 1C). However, by comparison of prometaphase and metaphase cells with anaphase cells (Figure 1D), it was clear that a larger fraction of merotelic kinetochores were producing anaphase lagging chromosomes in cells treated with 3 μM ZM447439 as compared to untreated cells (Figure 1D).

In untreated cells, an anaphase mechanism prevents formation of lagging chromosomes from merotelic kinetochore orientation when the two microtubule bundles connected to the merotelic kinetochore are significantly different in size [6]. To test whether this mechanism functioned properly in the presence of 3 μM ZM447439, we performed time-lapse spinning-disk confocal microscopy on PtK1 cells microinjected with Alexa488-labeled-CENP-F antibodies (to visualize kinetochores) and Rhodamine-labeled tubulin (to visualize kinetochore fibers) (Figure 2). In the presence of 3 μM ZM447439, about 50% of merotelic kinetochores analyzed by live-cell imaging produced anaphase lagging chromosomes, as opposed to 8% in untreated cells [6, 15]. Correct segregation of chromosomes with merotelically oriented kinetochores occurred when the fluorescence of the kinetochore fiber to the correct pole was significantly higher than the fluorescence of the fiber to the incorrect pole (Figure 2A, Movie S1). When the ratio was near 1, the chromosome lagged near the spindle equator

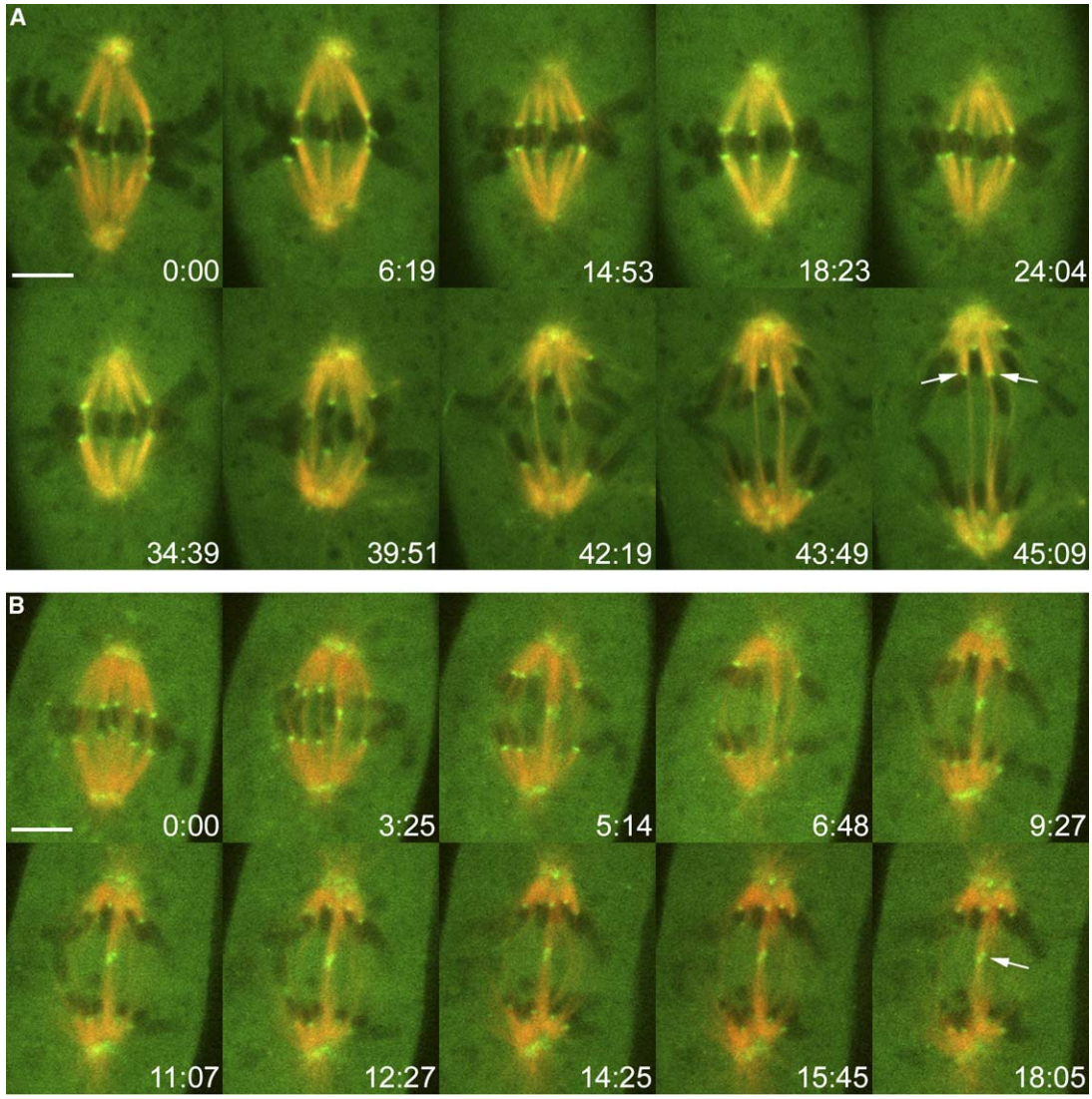

Figure 2. Aurora Kinase Inhibition Does Not Interfere with the Anaphase Mechanism that Prevents Missegregation of Merotelic Chromosomes

PtK1 cells treated with 3 μM ZM447439 were microinjected with A488-anti-CENP-F antibodies (kinetochores, green) and Rhodamine-tubulin (microtubules, red) and imaged by time-lapse, spinning-disk confocal microscopy.

(A) PtK1 cell possessing two merotelic kinetochores (arrows) that segregate to the spindle pole connected to the thicker microtubule bundle. The fluorescence intensity ratios for these two kinetochores were 3 and 2.2 (see Movie S1).

(B) PtK1 cell possessing two merotelic kinetochores, one of which can be followed throughout Movie S2 as it stays in focus in the majority of the frames. The fluorescence intensity ratio for this kinetochore was close to 1 (1.1), and, as expected, it produced a lagging chromosome during anaphase (see Movie S2). The scale bars represent 5 μm.

(Figure 2B, Movie S2). Measurements from deconvolved images of metaphase cells and live images of anaphase cells showed that the average ratio of kinetochore fiber fluorescence to the correct versus incorrect pole was ~1.5 in ZM447439-treated cells as opposed to ~2.5 in untreated cells (Table S1). Such a lower ratio for ZM447439-treated cells resulted from a larger number of merotelic kinetochores whose fluorescence intensity ratio was closer to 1 compared to control cells. Because a ratio near 1 produces missegregation of the merotelic kinetochore [6, 15], this result explains the higher frequencies of anaphase lagging chromosomes observed both in fixed (Figure 1B) and live cells (Figure 2) treated with 3 μM ZM447439. Therefore, partial Aurora B inhibition did not affect the ability of anaphase spindle mechanics to properly segregate chromosomes with merotelic kinetochores when the ratio of microtubules to the correct versus incorrect pole was sufficiently high. This is not surprising considering that Aurora B relocalizes from the centromere to the spindle midzone in anaphase [14], thus leaving the region where it could potentially regulate kinetochore-microtubule dynamics and chromosome movement. Instead, partial Aurora B inhibition greatly enhanced the number of kinetochores entering anaphase with microtubule fluorescence ratios near 1, the ratio that produces the highest frequency of lagging chromosomes. Thus, Aurora B plays a major role in the preanaphase correction mechanism that reduces the number of microtubules to the incorrect pole.

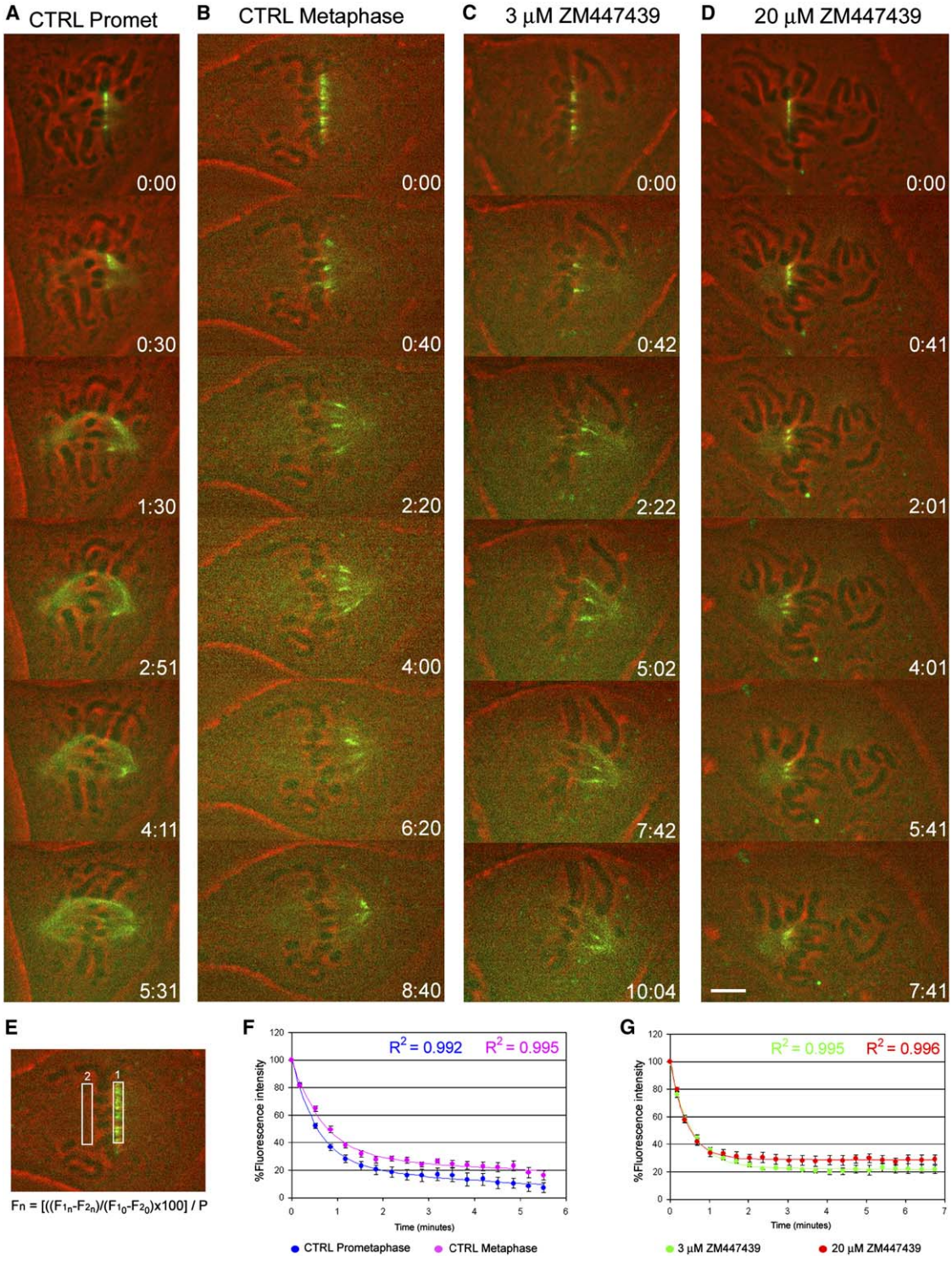

Figure 3. Aurora Kinase Inhibition Affects Both Kinetochore-Microtubule Turnover and Poleward Flux

(A)–(D) Tubulin photoactivation under different conditions in PtK1 cells expressing photoactivatable GFP-tubulin (PA-GFP-tubulin). The images at later time points were contrast-enhanced to clearly show the stable marks on kinetochore fibers. Nonenhanced QuickTime movies (Movies S3–S6) of these cells are available with the Supplemental Data. The scale bar represents 5 μm.

(A) Tubulin photoactivation in an untreated prometaphase cell. The images show that the photoactivated marks on kinetochore-microtubule fibers quickly reach the spindle pole in prometaphase cells. In addition, the photoactivated tubulin quickly becomes reincorporated into previously unlabeled regions of the mitotic spindle, thus brightening up the whole spindle.

(B) Tubulin photoactivation in an untreated metaphase cell. The slower microtubule poleward flux can be visualized as the photoactivated marks reach the spindle pole later than in prometaphase cells.

(C) Late-prometaphase cell treated with 3 μM ZM447439, exhibiting slower microtubule poleward flux (0.3 μm/min).

Kinetochore-Microtubule Turnover and Poleward Flux in Prometaphase Are Sensitive to Aurora Kinase Inhibition

The above results suggest that Aurora kinase inhibition produces stabilization of kinetochore microtubules, thus reducing the rate of detachment of kinetochore microtubules to the incorrect pole. This would increase the frequency of anaphase lagging chromosomes by producing more merotelic kinetochores entering anaphase with lower ratios of microtubules to correct versus incorrect pole. To test directly whether Aurora kinase inhibition stabilizes kinetochore-microtubule attachment, we obtained measurements of fluorescence dissipation after photoactivation (FDAPA) for kinetochore fibers in PtK1 cells stably expressing photoactivatable GFP-tubulin (PA-GFP-tubulin; [16]). We analyzed untreated prometaphase and metaphase cells and ZM447439-treated cells both at 3 μM and at the higher 20 μM concentration. We photoactivated a bar-shaped region within the spindle, so that the activated bar was more or less perpendicular to the long axis of the mitotic spindle and adjacent to the mass of chromosomes at the metaphase plate [16, 17] (Figures 3A–3D). Cells were imaged before and after photoactivation by time-lapse, spinning-disk confocal microscopy and phase-contrast microscopy (Figures 3A–3D; Movies S3–S6).

In untreated cells, photoactivated fluorescent marks on kinetochore microtubules persist longer than marks on nonkinetochore microtubules, and free tubulin activated inside the bar region diffuses away within seconds [17–21]. Measurements of fluorescence dissipation were performed as described by Zhai et al. [17] and diagrammed in Figure 3E. Measurements of fluorescence intensity minus background were obtained just after photoactivation and at intervals thereafter for each cell. The measured values were corrected for photobleaching by using measurements of photoactivated spindles with microtubules stabilized by 10 μM Taxol [22]. Plots were generated for each experimental condition by averaging at each time point corrected data obtained from 5–14 cells. With and without Aurora B inhibition, the kinetics of fluorescence dissipation after photoactivation were well fit by a double exponential curve [$F = A1 \times \exp(-k1 \times t) + A2 \times \exp(-k2 \times t)$], where A1 and A2 are the percentage of the total fluorescence contributed by nonkinetochore and kinetochore microtubules, k1 and k2 are their respective rate constants of turnover, and t is time after photoactivation (Figures 3F and 3G and Table 1). Regardless of the treatment, nonkinetochore microtubules within the photoactivated region represented about 70% of the fluorescence and exhibited a half-life of about 20 s, as expected [17, 23] (Table 1). The more stable kinetochore microtubules were about 30% of the microtubule population within the mark region (Table 1). Kinetochore-microtubule half-life depended on the stage of mitosis and on Aurora kinase inhibition. We found that the half-life for kinetochore microtubules in mid-to-late prometaphase was about 3 min (Figure 3F and Table 1), and it increased to about 7 min in metaphase cells (Figure 3F and Table 1). We did not measure microtubule turnover in anaphase, but Zhai et al. [17] reported similar values to ours for metaphase and a much longer half-life in anaphase (~35 min), a time when Aurora B has left the centromere. The double-exponential curve fit predicted high stability for kinetochore microtubules after treatment with either 3 μM or 20 μM ZM447439 (Figure 3G and Table 1). The estimated half-life in ZM447439-treated cells is clearly an approximation, considering that our measurements were performed over a period of 6–7 min, a period limited by flux of the marks into the pole. At 20 μM ZM447439, the standard error of regression was large for the kinetochore-microtubule fraction, indicating variation in the stability of kinetochore microtubules between different cells. We do not know the origin of this variability, but it might depend on the disruption, reported to occur with substantial Aurora B inhibition, of kinetochore protein assembly [24]. Nevertheless, our data clearly indicate that kinetochore-microtubule attachments become much more stable after Aurora kinase inhibition, and they represent the first measurement of the effect of Aurora kinase inhibition on kinetochore-microtubule stability in higher eukaryotes.

Ganem et al. [25] recently proposed that poleward flux of kinetochore microtubules prevents anaphase lagging chromosomes, perhaps by providing a sustained pulling force for microtubule detachment from kinetochores. We measured poleward-flux velocity in our photoactivation studies (Figures 3A–3D) by determining the distance between the edge of the fluorescent marks on kinetochore fibers and their spindle pole at each time point. Poleward flux was slower in metaphase as compared to prometaphase cells (0.57 ± 0.23 μm/min versus 0.93 ± 0.39 μm/min; Table 1). In cells treated with either 3 μM or 20 μM ZM447439, poleward flux was partially reduced (~0.4 μm/min) when compared to both prometaphase (t test, $p < 0.001$) and metaphase (t test, $p < 0.05$) control values (Table 1). It is possible that the decrease

(D) Cell treated with 20 μM ZM447439 and exhibiting a much slower microtubule poleward flux (0.2 μm/min). In all four columns it is possible to appreciate that the fluorescent marks move into the pole at later time points, so that both poleward flux and FDAPA can only be measured for a limited period of time.

(E) Diagram describing how fluorescence dissipation after photoactivation was measured in independent experiments. The fluorescence intensity at each time point was calculated with the formula shown and expressed as a percentage of the initial fluorescence. At each time point, the fluorescence intensity was corrected for photobleaching (P), which was calculated as the decrease over time in the fluorescence of a mark generated in Taxol-treated cells.

(F) Fluorescence dissipation after photoactivation in control PtK1 cells.

(G) Fluorescence dissipation after photoactivation in ZM447439-treated PtK1 cells exhibiting increased stability of kinetochore microtubules. In (F) and (G), the filled circles represent the average values recorded at each time point after photoactivation for 5–14 different cells. The bars represent the standard error of the average. The line shows the double exponential curve generated by nonlinear curve fitting with Sigma Plot software and the function $F = A1 \times \exp(-k1 \times t) + A2 \times \exp(-k2 \times t)$, where A1 and A2 are the fraction of microtubules with turnover rate constants k1 and k2, and t is time after photoactivation. Nonkinetochore microtubules turn over rapidly with half-lives of about 20 s, independent of ZM447439 treatment or mitotic stage. Kinetochore microtubules turn over more slowly at a rate that slows down with mitotic progression and ZM447439 treatment. R^2 values for each fit are reported at the top right corner of the graph.

Table 1. Summary of Kinetochore-Microtubule Poleward Flux and Turnover in PtK1 Cells Expressing Photoactivatable GFP-Tubulin

	Microtubule Turnover						Microtubule Poleward Flux	
	Non-Kinetochore MTs[a] (average ± SE[b])		Kinetochore MTs (average ± SE[b])					
	%	$T_{1/2}$ (sec)	%	$T_{1/2}$ (min)	R^2	N	Average ± SD (μm/min)	N
Control Prometaphase	75 ± 4	17.0 ± 1.9	27 ± 4	3.5 ± 0.8	0.992	6–12	0.93 ± 0.39	15
Control Metaphase	68 ± 3	21.5 ± 1.8	32 ± 3	7.4 ± 1.7	0.995	7–14	0.57 ± 0.23	17
3 μM ZM447439	75 ± 2	22.2 ± 1.2	23 ± 1	52.6 ± 50.4	0.995	5–13	0.42 ± 0.16	14
20 μM ZM447439	72 ± 1	17.2 ± 0.8	29 ± 1	230.0 ± 565.6	0.996	6–10	0.38 ± 0.19	14

[a] MTs denotes microtubules.
[b] Standard errors from regression analysis of the average fluorescence dissipation values.

in kinetochore-microtubule flux rate we detected was produced by inhibition of Aurora A kinase activity, rather than Aurora B, because Aurora A is concentrated at spindle poles [26]. Although reduced by ZM447439 treatment, kinetochore-microtubule poleward flux still occurred at a significant rate as opposed to the inhibition reported by Ganem and coworkers [25] after depleting Kif2a and MCAK.

We finally measured average centromere stretch between sister kinetochores to test whether the reduced poleward-flux velocity changed the average tension at kinetochores of metaphase chromosomes. Although 3 μM ZM447439 somewhat suppressed the chromosome oscillation that normally takes place in the middle of the spindle (Figure S4), we did not find a significant difference in average centromere stretch between control and 3-μM-ZM447439-treated cells for chromosomes that oscillated back and forth in the middle of the spindle (1.85 ± 0.34 versus 1.87 ± 0.23 μm) and for chromosomes that exhibited little oscillation at the spindle periphery [6, 16] (2.06 ± 0.39 versus 1.95 ± 0.16 μm).

We conclude that the increase in merotelic kinetochore orientation after inhibition of Aurora kinase is mostly a consequence of the increased stability of kinetochore microtubules and that Aurora B plays a critical role in destabilizing kinetochore-microtubule attachments in mammalian tissue cells. In addition, partial Aurora kinase inhibition produces a major reduction in the rate of turnover of kinetochore microtubules in prometaphase and metaphase without changing kinetochore tension. These results indicate that regulation of kinetochore-microtubule attachment by Aurora B occurs downstream from tension regulation [9, 27, 28]. In addition, they suggest that attachment stability and tension generation involve independent interfaces at the kinetochore rather than a single interface between the kinetochore attachment proteins and the microtubule lattice [15].

A Model for How Tension and Aurora B Kinase Activity Work Together to Stabilize Correct Attachments and Destabilize Incorrect Ones

In Figure 4, we propose a model for correction of merotelic attachments in which Aurora B plays a critical role in promoting microtubule destabilization. In our model, attachment sites in the kinetochore outer plate are exposed to a destabilization gradient produced by the kinase activity of Aurora B bound to or recently dissociated from the inner centromere (centromere-associated Aurora B turns over with a $t_{1/2}$ = 47 ± 24 s [29]), which we term Aurora B*. For chromosomes with unattached sister kinetochores in early prometaphase, the centromere is not stretched and microtubule attachment is rapidly destabilized because of proximity to higher concentrations of Aurora B* (Figure 4A). When chromosomes become bioriented and achieve amphitelic kinetochore orientation, the centromere becomes stretched by kinetochore-microtubule pulling force. This moves attachment sites away from the higher concentrations of Aurora B* at the inner centromere (Figure 4B) and reduces the probability of detachment. In support of this mechanism, we found that the half-life of kinetochore microtubules in early to midprometaphase was about 3 min, whereas at metaphase, when kinetochores achieve a full complement of kinetochore microtubules and become stretched, the half-life was about 7 min. In addition, this mechanism explains the stability of kinetochore-microtubule attachment in anaphase (~35 min [17]), when Aurora B leaves the centromere to relocalize to the spindle midzone [14], and Aurora B* is not close to the site of kinetochore-microtubule attachment to promote destabilization. This mechanism also explains how tension promotes attachment stability for correct attachments before anaphase as discovered by Nicklas and colleagues [27]: the higher the tension, the further kinetochore-microtubule attachment sites become from the region of high Aurora B*, the less microtubule-kinetochore attachments will be destabilized. Finally, it explains how incorrect attachments at merotelic kinetochores could be preferentially destabilized and correct attachments stabilized (Figure 4C). For merotelic kinetochores of chromosomes aligned at the metaphase plate, only the attachment sites connected to the incorrect pole are positioned close to the region of high Aurora B*, whereas correctly attached sites are pulled away (Figure 4C). Correction of misattachment requires two steps: detachment of misattached microtubules from the incorrect pole (Figure 4D) and reattachment of the free attachment sites to the correct pole (Figure 4E). Tension toward the correct pole stretches the kinetochore, producing stability for correct attachments, whereas tension on the incorrect attachments pulls them toward the center of the centromere, promoting instability. Misattached microtubules will detach at a certain rate that is determined by the position in the Aurora B* gradient determined, in turn, by the tension at each attachment site. Our results show that when Aurora B is inhibited, kinetochore-microtubule turnover becomes much slower (Figure 3 and Table 1). This would reduce the probability for turnover of microtubules to

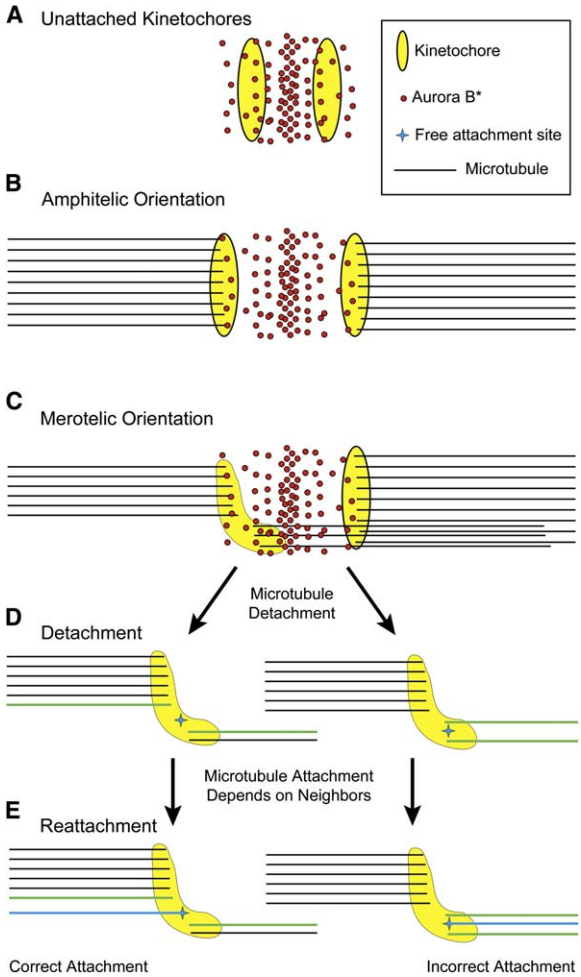

Figure 4. Aurora B Contributes to Correction of Merotelic Attachments by Promoting Microtubule Destabilization

A destabilization gradient is produced by the kinase activity of Aurora B bound or recently dissociated from the inner centromere (Aurora B*).

(A) For unattached chromosomes in early prometaphase, the centromere is not stretched and most attachment sites lay close to the region where Aurora B* is higher. As a consequence, kinetochore-microtubule turnover is fast.

(B) When the chromosome achieves amphitelic orientation and microtubule attachment sites are pulled away from the Aurora B*-enriched region, microtubule attachment becomes more stable (i.e., slower turnover).

(C) For merotelic kinetochores, the attachment sites connected to the incorrect pole are positioned close to the region of high Aurora B*, which can thus induce microtubule destabilization and leave empty attachment sites (blue stars in [D]).

As diagrammed in (D) and (E), the probability of reattachment to the correct or incorrect pole depends on the nearest neighbors (microtubules highlighted in green). If microtubules on either side are to one pole, then it will be probable that the attachment will be to a microtubule (blue) from the same pole ([E], right). If nearby attachments are to opposite poles ([D], left), then the new attachment (blue microtubule) will be equally likely to be to one pole or the other (attachment to the correct pole is shown in the figure). (See Results and Discussion for details.)

the wrong pole, inhibiting correction of merotelic kinetochore orientation and producing the high levels of anaphase lagging chromosomes we observed in Aurora B inhibited cells. If a detachment occurs, the probability of reattachment to the correct or incorrect pole likely involves constraints from attachment of nearest neighbors (compare left and right panels of Figures 4D and 4E). If microtubules on either side are attached to one pole, then it will be probable that a new attachment will be to the same pole (Figure 4D and 4E, right). If nearby attachments are to opposite poles, then the new attachment will be equally likely to be to one pole or the other (Figure 4D and 4E, left). These nearest-neighbor rules provide an explanation for why merotelic kinetochores can persist into late metaphase for longer than 60 min [3] despite a kinetochore-microtubule half-life of 3–7 min.

Conclusions

Inaccurate chromosome segregation produces aneuploidy, a hallmark of cancer and a major cause of miscarriage, stillbirth, and genetic diseases. An important discovery of our study is that kinetochore-microtubule stability and accuracy of chromosome segregation in mammalian tissue cells are very sensitive to partial inhibition of Aurora B kinase activity. The sensitivity indicates that mutations in Aurora B—or in proteins that control Aurora B—that do not completely block kinase activity, may have a great impact on phosphorylation of targets that control kinetochore-microtubule stability and chromosome segregation in vivo, but not be detected by in vitro phosphorylation assays of standard substrates as we have found for histone H3.

The identification of Aurora B targets at the centromere is still under way. However, kinetochore-microtubule stability could be regulated by several candidate proteins, including Ndc80, the DAM/DASH complex, MCAK, and others. The kinetochore protein Ndc80 and the DAM/DASH complex are part of the protein complex involved in the kinetochore-microtubule linkage in budding yeast and interact with Ipl1 (yeast Aurora) [9, 30, 31]. In addition, Hec1 (human Ndc80) has recently been found to be a target for Aurora B-dependent phosphorylation (J.G. DeLuca and E.D.S., unpublished data). Preventing the microtubule depolymerase MCAK from localizing to the centromere is known to greatly enhance the incidence of anaphase lagging chromosomes [10]. Because the depolymerase activity of MCAK is reduced by Aurora B phosphorylation [32–34], partial inhibition of Aurora B should promote MCAK activity. Clearly, this seems to be a paradox; it is not obvious how enhanced MCAK depolymerase activity would result in more stable kinetochore-microtubule attachments. However, regulation of MCAK by Aurora B could be determined by the state of specific centromere and kinetochore subregions and could vary within individual chromosomes, because both proteins appear enriched at *Xenopus* S3 cell centromeres with merotelic attachments (see Knowlton et al. in this issue of *Current Biology* [35]).

Supplemental Data

Supplemental Data include Experimental Procedures, four figures, one table and six movies and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/17/1711/DC1/.

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