Cells satisfy the mitotic checkpoint in Taxol, and do so faster in concentrations that stabilize syntelic attachments

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Introduction

During cell division the mitotic checkpoint minimizes aneuploidy by delaying anaphase and exit from mitosis until all kinetochores are stably attached to microtubules (MTs). When the checkpoint cannot be satisfied, i.e., in the presence of one or more unattached kinetochores, many human cells escape mitosis after a prolonged (~20 h) delay to form tetraploid G1 cells. During this process, termed mitotic slippage, the cyclin B subunit of the cyclin B/CDK1 kinase is slowly destroyed in an APC-dependent manner in the presence of an active checkpoint. As a result, over time cyclin B/CDK1 activity falls below that needed to maintain the mitotic state (Brito and Rieder, 2006).

Taxol is a MT-stabilizing drug currently used to treat various cancers. Although its mode of action is unknown, entry into mitosis is required for Taxol sensitivity (Sudo et al., 2004) where it is thought to induce apoptosis by inhibiting mitotic checkpoint satisfaction. Yet, when cultured human cells are treated with clinically relevant Taxol concentrations (5–10 nM) mitosis is not arrested. Rather, after a few hours the cells satisfy the checkpoint and complete division to produce 2–3 daughters (Ikui et al., 2005), many of which (depending on the cell type) die in G1 (Brito and Rieder, 2009). However, at concentrations between 50 and 100 nM, Taxol is widely reported to arrest cells in mitosis until they die or escape via mitotic slippage (Gascoigne and Taylor 2008; Shi et al., 2008).

We recently found that when mitotic checkpoint satisfaction is prevented with nocodazole or Eg5 inhibitors, human telomerase-immortalized RPE1 cells average ~20 h in mitosis before slipping into G1. At the same time, however, we noted that in 500 nM Taxol RPE1 averaged just 12 h in mitosis, and this shortened duration of mitosis (DM) could not be attributed to MT assembly, which occurs also in Eg5 inhibitors. We therefore hypothesized that RPE1 cells ultimately satisfy the checkpoint in Taxol and do so faster at concentrations >0.5 µM. Inhibiting the aurora-B kinase in Taxol-treated RPE1 cells accelerates checkpoint satisfaction by stabilizing syntelic kinetochore attachments and reduces the DM to ~1.5 h regardless of drug concentration. A similar stabilization of syntelic attachments by Taxol itself appears responsible for accelerated checkpoint satisfaction at concentrations >0.5 µM. Our results provide a novel conceptual framework for how Taxol prolongs mitosis and caution against using it in checkpoint studies. They also offer an explanation for why some cells are more sensitive to lower versus higher Taxol concentrations.

To determine why the duration of mitosis (DM) is less in Taxol than in nocodazole or Eg5 inhibitors we studied the relationship between Taxol concentration, the DM, and the mitotic checkpoint. We found that unlike for other spindle poisons, in Taxol the DM becomes progressively shorter as the concentration surpasses ~0.5 µM. Studies on RPE1 and PtK2 expressing GFP/cyclin B or YFP/Mad2 revealed that cells ultimately satisfy the checkpoint in Taxol and do so faster at concentrations >0.5 µM. Inhibiting the aurora-B kinase in Taxol-treated RPE1 cells accelerates checkpoint satisfaction by stabilizing syntelic kinetochore attachments and reduces the DM to ~1.5 h regardless of drug concentration. A similar stabilization of syntelic attachments by Taxol itself appears responsible for accelerated checkpoint satisfaction at concentrations >0.5 µM. Our results provide a novel conceptual framework for how Taxol prolongs mitosis and caution against using it in checkpoint studies. They also offer an explanation for why some cells are more sensitive to lower versus higher Taxol concentrations.

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Results and discussion

For drugs that prevent MT assembly or centrosome separation, the DM increases with concentration to a point after which it no longer changes. For HeLa this point ranges from 6 nM in vinblastine to 100 nM in nocodazole (Jordan et al., 1992), and 1.5 µM in S-Trityl-l-cysteine (an Eg5 inhibitor; Skoufias et al., 2006). Here, we report that this relationship does not exist for Taxol and its analogues: although the DM in RPE1 cells treated with 5, 50, or 500 nM Taxol increased from 2.5 h to 12 h, in 5 and 10 µM Taxol mitosis averaged, respectively, just 5 and 3.5 h and 99% of the cells survived (Fig. 1 B; Table S1). The same was true for the Taxol analogue epothilone B (Fig. 1 C). This hitherto unreported response to Taxol was also seen in normal human BJ fibroblasts and rat kangaroo kidney (PtK2) cells, and to a lesser extent in HeLa and U2OS (Fig. 1, D–F; Table S1). Thus, unlike drugs that inhibit MT assembly or centrosome separation, the dose–response curve for the DM in drugs that promote MT stability is biphasic, increasing to a point after which it decreases.

Accelerated exit from mitosis in Taxol concentrations >0.5 µM is due to a drug effect on spindle microtubules

Why is the DM in 5 µM Taxol less than in 0.5 µM Taxol? One idea is that at high concentrations Taxol and epothilone B have off-site target(s) independent of MTs. To test this we treated RPE1 for 3 h with 3.2 µM nocodazole to prevent MT assembly, and then added 5 µM Taxol. We reasoned that if in the absence of MTs 5 µM Taxol still drives cells from mitosis in <5 h, then there is an off-site target involved. However, even in 3.2 µM nocodazole 5 µM Taxol stimulated MT assembly (unpublished data). We therefore modified this experiment by locking tubulin into paracrystals with 10 µM vinblastine before adding 5 µM Taxol. Under this condition MT formation was inhibited and the cells remained in mitosis for >27 h, similar to after vinblastine treatment alone (Fig. 1 G; Table S1). Thus, the DM is reduced in Taxol concentrations >0.5 µM because of how the drug influences spindle MTs.

Cells satisfy the mitotic checkpoint in Taxol and do so more rapidly at high concentrations

Another idea for why the DM versus concentration plot in Taxol is biphasic is that cells satisfy the checkpoint in Taxol, but do so faster at high concentrations. This is suggested by our data that the DM in 5 nM to 10 µM Taxol is much less than the 20 h seen in cells that cannot satisfy the checkpoint (above). The unchallenged assumption that, as for agents that inhibit MT assembly, Taxol at >100 nM prevents checkpoint satisfaction comes from reports that at these concentrations: (1) spindle assembly is
grossly perturbed, (2) cells are delayed in mitosis, and (3) kinetochores are always present in fixed cells that are positive for the Mad2 checkpoint protein (Waters et al., 1998). However, the checkpoint detects unattached kinetochores, not perturbations in spindle assembly, and it can be satisfied on grossly abnormal spindles (Iki et al., 2005; Brito et al., 2008). Also, exit from mitosis due to checkpoint satisfaction differs mechanistically from slippage (Brito and Rieder, 2006). Finally, cells lacking Mad2 at kinetochores would rarely be encountered in Taxol-treated cultures because as this condition occurs the cells rapidly exit mitosis.

Mad2 binds to unattached kinetochores (Chen et al., 1996) and is lost from kinetochores as they acquire MTs (Waters et al., 1998; Nicklas et al., 2001). Because checkpoint satisfaction coincides with loss of Mad2 from all kinetochores (Hoffman et al., 2001), we asked if Mad2 is ultimately lost from all kinetochores in Taxol. To answer this we treated RPE1 for 12 h (the average DM in 0.5 µM Taxol) with 0.5 or 10 µM Taxol and then added a proteasome inhibitor (10 µM MG132) 2 h before fixation and Mad2 staining. We reasoned that if in Taxol cells satisfy the checkpoint before exiting mitosis, then preventing exit should enrich for cells lacking Mad2 at all kinetochores. Indeed, although most cells in each concentration exhibited varying degrees of kinetochore-Mad2 staining, cells could be readily located in both in which all kinetochores lacked Mad2 (Fig. S1). These indirect data suggest that cells satisfy the checkpoint in 0.5–10 µM Taxol before escaping mitosis.

To directly verify this we followed PtK2 stably expressing Mad2/YFP (Mad2/PtK2) as they entered mitosis in 0.5 or 20 µM Taxol. The DM in these cells, i.e., the period from nuclear envelope breakdown (NEB) to the initiation of cytokinesis, was 45 ± 8 min (n = 40), and the period between loss of Mad2/YFP on the last kinetochore and the start of cytokinesis was 17 ± 3 min (n = 16). In 0.5 µM Taxol Mad/PtK2 cells averaged 140 ± 39 min (n = 92) in mitosis versus 97 ± 25 min (n = 54) in 20 µM Taxol. We found, in all cases and in both drug concentrations, that Mad2/PtK2 remained in mitosis until the last kinetochore had stably lost its Mad2 signal, ~20 min after which an aborted cytokinesis began (Fig. 2, A–C). Also, in both concentrations 85–90% of the kinetochores lost their Mad2 staining within 40 min of NEB, so that the checkpoint was maintained by those few that required longer to become stably depleted of Mad2 (Fig. 2, D–G). From these direct data we conclude that exit from mitosis in Taxol-treated PtK2 cells occurs over a broad (0.5–20 µM) range of concentrations from checkpoint satisfaction.

Checkpoint satisfaction can also be inferred from a sudden steep drop in cyclin B/GFP fluorescence intensity just before (Chang et al., 2003) or at (Clute and Pines, 1999) chromatid disjunction (Fig. 3, A and D). We therefore followed GFP fluorescence intensity in RPE1 transiently expressing low levels of cyclin B/GFP as they entered mitosis in 0.5 to 10 µM Taxol. These data were then analyzed to determine if cyclin B/GFP decreased at a “background” level until the cell slipped through mitosis (Brito and Rieder, 2006), or if it slowly decreased as during slippage but then rapidly fell just before mitotic exit as for checkpoint satisfaction in 5 nM Taxol (Brito et al., 2008). In all five cells examined in both 5 and 10 µM Taxol, mitotic exit occurred shortly after a sudden precipitous drop in cyclin B/GFP fluorescence (Fig. 3, B and D). This result confirms that, as for PtK2, RPE1 exit from mitosis in ≥5 µM Taxol is due to checkpoint satisfaction.

Although our Mad2 data (Fig. S1) suggest that like PtK2, RPE1 also satisfy the checkpoint in 0.5 µM Taxol, attempts to demonstrate this by cyclin B/GFP degradation gave results difficult to interpret (Fig. 3 D). However, as for other cell types, in a given spindle poison the DM in RPE1 is highly variable: e.g., 744 ± 399 min for 0.5 µM Taxol and 1218 ± 424 min in dimethyl sulfoxide (Brito et al., 2008). Thus, although some cells may slip through mitosis in 0.5 µM Taxol before checkpoint satisfaction, the significantly reduced average DM in 0.5 µM Taxol (12 h) compared with when the checkpoint cannot be satisfied (20 h) suggests that most RPE1 satisfy the checkpoint in 0.5 µM Taxol.

As previously reported for PtK (Waters et al., 1998), we found no tension on centromeres in PtK2 or RPE1 dividing in 0.5–10 µM Taxol (Fig. S2). However, in 10 µM Taxol the cells satisfy the mitotic checkpoint in just several hours. Thus, as concluded recently by others (O’Connell et al., 2008; Uchida et al., 2009), production of the checkpoint signal is not due to a lack of tension on the centromere. That is, there is no centromere-based Mad2-independent tension-sensing pathway for maintaining the checkpoint in the absence of tension between sister kinetochores (Skoufias et al., 2001; Ahonen et al., 2005).

In Taxol, checkpoint satisfaction is delayed until all monotelic and syntelic attachments are converted into amphitelic or stable syntelic attachments

The spindle assembly pathway in Taxol differs from that in untreated cells: in the latter, kinetochore attachment occurs over 15–20 min (Fig. 2 A) from a search-and-capture process, dependent on MT dynamics which is facilitated by the motion of chromosomes and asters (Kapoor et al., 2006; Cai et al., 2009). By contrast, as cells enter mitosis in >100 nM Taxol they form many small asters that migrate at NEB to the chromosomes, where they form a large multipolar spindle (Maekawa et al., 1991; Hornick et al., 2008) (Fig. 4 A). Under this condition astral MTs are substantially shorter, their dynamics are depressed, and the chromosomes are immobile. As a result, the rate kinetochores attach to MTs in Taxol is retarded because it is defined by the movement of the asters and not the dynamic behavior of their associated MTs or chromosomes.

As in a normal mitosis, in Taxol many chromosomes become attached to an aster in a monotelic or syntelic fashion (Fig. 4, A, F, and G, arrowheads). In untreated cells, syntelic attachments are corrected by a constitutive mechanism involving chromosomal passenger complexes, composed of aurora B, survivin, borealin, and INCENP, located in the centromere (Ruchaud et al., 2007). The correction of a syntelic attachment leads to the transient production of a monotelic chromosome with an unattached (and thus checkpoint signaling) kinetochore (Tanaka et al., 2002; Pinsky et al., 2006). In cells containing many syntelic chromosomes but MTs with normal dynamics, as on monastrol spindles formed in Eg5 inhibitors (Kapoor...
Figure 2. In Taxol, Mad2 is progressively depleted from kinetochores until the checkpoint is satisfied (see also Fig. S1). (A–C) YFP/Mad2-PK2 cells were followed without treatment (A) or in 0.5 (B) or 20 µM (C) Taxol. Top rows are phase-contrast images and bottom rows are maximum intensity projected YFP fluorescence images of the same cell. Exit from mitosis occurs only after the last Mad2-positive kinetochore (A and C, arrowhead) loses its Mad2. (B) In 0.5 µM Taxol a kinetochore (arrowhead) can lose and then reacquire Mad2, as shown in high-magnification images from the boxed regions. Membrane blebbing is typical as cells exit mitosis in Taxol (C) and is not due to apoptosis. (D) Time vs. average number of Mad2-positive kinetochores in controls and after treatment with 10 µM nocodazole or 20 µM Taxol. (E–G) Similar plots of individual controls (E), or cells treated with 0.5 µM (F) or 20 µM Taxol (G). The number of Mad2-positive kinetochores often transiently increases in 0.5 µM but not 20 µM Taxol.
checkpoint satisfaction is prevented because error correction promotes a continuous cycling between syntelic (nonsignaling) and monotelic (signaling) states (Lampson et al., 2004). As a result, killing the correction mechanism in these cells with aurora B inhibitors rapidly stabilizes all syntelic attachments (Hauf et al., 2003; Lampson et al., 2004; Loncarek et al., 2007), which leads to Mad2 depletion at all kinetochores and checkpoint satisfaction (Hauf et al., 2003; Lens et al., 2003). As in Eg5 inhibitors, the checkpoint cannot be satisfied in Taxol until the asters have gathered all the chromosomes, and then not until all monotelic attachments are converted either into “normal” amphitelic attachments or into syntelic attachments that are somehow stabilized. Although the former process is retarded by the Taxol-induced reduction in aster MT dynamics and inhibition of chromosome motility, the latter is predicted to be promoted by the stabilizing effects of Taxol on kinetochore MTs.

Stabilizing syntelic attachments in Taxol accelerates exit from mitosis by promoting mitotic checkpoint satisfaction
As on monastrol spindles (above), inhibiting aurora B in Taxol-treated cells accelerates exit from mitosis. We find, in fact, that inhibiting aurora B in RPE1 treated with 0.5 µM Taxol reduces the DM by ~90% (from 12 to 1.5 h; Table S1). An obvious explanation for this is that as in Eg5 inhibitors, inhibiting aurora B in Taxol promotes checkpoint satisfaction by stabilizing syntelic attachments. However, others have concluded that inhibiting aurora B drives cells treated with Taxol or Eg5 inhibitors from mitosis because its activity is required for a functional mitotic checkpoint. Although the supporting data are indirect, the
However, one can argue that the nocodazole concentrations used (330–660 nM) were insufficient to completely disrupt MT assembly, and instead promoted the formation of monopolar...
spindles containing up to 75% of the normal MT mass (Brinkley et al., 1967; Jordan et al., 1992).

If aurora B activity is required for the mitotic checkpoint, then inhibiting it in cells lacking MTs should induce exit from mitosis in ~30 min, as when bona fide checkpoint components are depleted or inhibited. To test this, we followed RPE1 cells entering mitosis in 3.2 µM nocodazole and a Hesperadin concentration (100 nM) that inhibits aurora B (Hauf et al., 2003). We found that these cells average 17 h in mitosis, similar to the 19 h seen after nocodazole treatment alone (Table S1), and that Mad2 accumulated on kinetochores in these cells to the same level as after nocodazole alone (Fig. 4, B–D). We also found that inhibiting aurora B did not prevent YFP/Mad2 from accumulating on kinetochores in nocodazole-treated PtK2 cells (Fig. 5 B), and when Hesperadin-treated metaphase PtK2 cells lacking Mad2 at most kinetochores were exposed to nocodazole, Mad2 rapidly reappeared at the kinetochores (Fig. 5 C). Together, these findings reveal that aurora B activity is not required for recruitment of Mad2 to kinetochores or for proper mitotic checkpoint function.

Mad2 also accumulates on kinetochores as Taxol (0.5–20 µM)-treated RPE1 (Fig. 4 E) and PtK2 (Fig. 5 D) cells enter mitosis in Hesperadin but, as for cells treated with Taxol only (Fig. 2, B and C), Mad2 is then progressively lost from all kinetochores after which the cells exit mitosis (Fig. 4, F and G; Fig. 5 D). Because the 90% reduction in the DM after inhibiting aurora B in Taxol-treated RPE1 cells is not due to a requirement for aurora B in the checkpoint (above), and because this accelerated exit does not occur in the presence of unattached (Mad2-positive) kinetochores, it must be due, as on monastrol spindles, to the depletion of free kinetochores via the stabilization of syntelic attachments (Fig. 4, F and G, arrowheads).

**Elevated Taxol concentrations accelerate checkpoint satisfaction by stabilizing syntelic kinetochore attachments**

In Taxol, progressive concentration increases stabilize MTs by inhibiting plus- and then minus-end MT dynamics in vitro (Derry et al., 1995, 1998) and in vivo (Jordan et al., 1993) until a point above which MT assembly is promoted. In HeLa, spindle MT mass begins to increase above 10 nM Taxol until it peaks at 330 nM, 50% above normal (Jordan et al., 1993). This is likely true for other cells, although the concentration for inducing maximum spindle MT mass may differ. Thus, in RPE1, checkpoint satisfaction may occur more rapidly in 5 vs. 0.5 µM Taxol because 5 µM promotes the formation of more MTs for recruitment to kinetochores. However, this cannot be the full story because in RPE1 increasing Taxol from 5 to 10 µM is unlikely to increase spindle MT mass, yet it decreases the DM from 5 to 3.5 h (Fig. 1).

As noted above, in Taxol the mitotic checkpoint cannot be satisfied until all kinetochores become stably attached to MTs, i.e., until existing monotelic and syntelic attachments are converted into amphibetic attachments or stable syntelic attachments. This being the case, an attractive explanation for why the DM is shorter in 5–10 µM versus 0.5 µM Taxol is that after a point, further concentration increases make it progressively more difficult for the error correction mechanism to produce free kinetochores from syntelic attachments. To test this we asked if, in 500 nM Taxol, Mad2 can suddenly and transiently reappear on a kinetochore after it had lost its Mad2, as would occur during the correction and reformation of a syntelic attachment. We found that this does indeed occur in 0.5 µM Taxol (Fig. 2 B) and it leads to transient increases in the number of Mad2-positive kinetochores (Fig. 2 F). That this behavior was not seen in 20 µM Taxol (Fig. 2 G) implies that the correction mechanism is attenuated at elevated Taxol concentrations.

One prediction of our model is that inhibiting aurora B in Taxol-treated cells should, by stabilizing syntelic attachments, lead to a similar DM regardless of the drug concentration, and this too is the case: inhibiting aurora B in RPE1 treated with 0.5 or 10 µM Taxol decreases the DM from, respectively, 12 and 3.5 h to just 1.5 h (Table S1). Under these conditions, exit from mitosis coincides both with the depletion of YFP/Mad2 from the last Mad2-positive kinetochore (Fig. 5 D) and a sudden, rapid increase in the rate of cyclin B degradation (Fig. 3, C and D), i.e., from checkpoint satisfaction.

In summary, our results support a model in which Taxol delays cells in mitosis until the last monotelic chromosome establishes a normal amphitelic connection to an adjacent aster, which satisfies the mitotic checkpoint. As the Taxol concentration increases this event is progressively delayed due to the progressive inhibition of chromosome motion and MT dynamics. Then, at some point a concentration is reached in which further increases accelerate checkpoint satisfaction by making it more difficult to generate free (signaling) kinetochores from syntelic attachments formed in the drug. Although it is unclear how high Taxol concentrations stabilize syntelic attachments, it is noteworthy that as syntely is corrected the kinetochore that loses its MT attachment to a spindle pole (aster) remains oriented toward that pole because external forces are not present to move it to the opposite side of the centromere (Loncarek et al., 2007). Thus, even if the error correction mechanism breaks the MT attachments on a given kinetochore in >0.5 µM Taxol, e.g., by preventing Hec1 from binding MTs (DeLuca et al., 2006), the attachment should rapidly reform as the old Taxol-stabilized kinetochore fiber MTs are re-bound.

**A reason why some cells are more sensitive to lower versus higher Taxol concentrations**

We found that although 27% of RPE1 cells died in or shortly after mitosis in 0.5 µM Taxol (Brito and Rieder, 2009), only 1% died in 5–10 µM Taxol—and the same was true for epothilone B (Table S1). Similarly, Gascoigne and Taylor (2008) reported that almost all H1703 cells die during mitosis in 100 nM Taxol, whereas in 10 µM Taxol 63% escape as viable cells. In an earlier but more thorough study, Yeung et al. (1999) noted that after 24 h in 50 nM Taxol the mitotic index in four of six breast cancer lines ranged from 66–82%, and by 48 h 59–79% of the cells had died by apoptosis. However, after 24 h in 25 µM Taxol these same lines exhibited both a significantly lower mitotic index (6–15%) and a dramatic reduction (4–12%) in apoptosis. Thus, unlike for other spindle poisons some cells are, paradoxically, more sensitive to lower versus higher Taxol concentrations.
finding that the DM in high Taxol concentrations is much shorter than in lower ones provides a potential explanation for the above paradox: for some cell types the delay in mitosis induced by 100–500 nM Taxol is long enough to trigger the death switch, whereas at higher concentrations the cells escape mitosis before this point.

In general, the longer the mitotic checkpoint delays a cell in mitosis the more likely it is to die during or after mitosis. This implies that a “death switch” is thrown at some point during the prolonged mitosis after which the cell is destined to die (Rieder and Maiato, 2004; Tao et al., 2005). This being the case, our finding that the DM in high Taxol concentrations is much shorter than in lower ones provides a potential explanation for the above paradox: for some cell types the delay in mitosis induced by 100–500 nM Taxol is long enough to trigger the death switch, whereas at higher concentrations the cells escape mitosis before this point.
normalizing the value from cells treated with nocodazole plus Hesperadin accordingly. Integrated intensities were measured by ImageJ and the results were processed by Microsoft Excel software.

Online supplemental material

Table S1 summarizes how the duration of mitosis changes in RPE1, BJ fibroblasts, HeLa, and U2OS in response to various drug treatments. Fig. S1 documents the progressive depletion of Mad2 from kinetochores in Taxol-treated RPE1 cells when exit from mitosis is inhibited with MG132. Fig. S2 documents the lack of tension on centromeres in Taxol-treated RPE1 and PK2 cells under conditions in which the mitotic checkpoint is satisfied. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200906150/DC1.

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References

Ahonen, L.J., M.J. Kallio, J.R. Daum, M. Bolton, I.A. Manke, M.B. Yaffe, P.T. Stutkenberg, and G.J. Gorbsky. 2005. Polo-like kinase 1 creates the tension-sensing 3F2 phosphoepitope and modules the association of spindle-checkpoint proteins at kinetochores. Curr. Biol. 15:1078–1089.

Brito, D.A., and C.L. Rieder. 2006. Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. Curr. Biol. 16:1194–1200.

Brito, D.A., and C.L. Rieder. 2009. The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. Cell Motil. Cytoskeleton. 66:437–447.

Brito, D.A., Z. Yang, and C.L. Rieder. 2008. Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. J. Cell Biol. 182:623–629.

Cai, S., C.B. O’Connell, A. Khodjakov, and C.E. Walczak. 2009. Chromosome congression in the absence of kinetochore fibres. Nat. Cell Biol. 11:832–838.

Chang, D.C., N. Xu, and K.Q. Luo. 2003. Degradation of cyclin B is required for the onset of anaphase in mammalian cells. J. Biol. Chem. 278:37865–37873.

Chen, R.-H., J.C. Waters, E.D. Salmon, and A.W. Murray. 1996. Association of spindle assembly checkpoint component XMD2 with unattached kinetochores. Science. 274:242–246.

Clute, P., and J. Pines. 1999. Temporal and spatial control of cyclin B destruction in metaphase. Nat. Cell Biol. 1:82–87.

DeLuca, J.G., W.E. Gall, C. Ciferri, D. Cimini, A. Musacchio, and E.D. Salmon. 2006. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. J. Cell Biol. 172:969–982.

Derry, W.B., L. Wilson, and M.A. Jordan. 1995. Substoichiometric binding of taxol suppresses microtubule dynamics. Biochemistry. 34:2203–2211.

Derry, W.B., L. Wilson, and M.A. Jordan. 1998. Low potency of taxol at microtubule minus ends: implications for its antimotic and therapeutic mechanism. Cancer Res. 58:1177–1184.

Ditchfield, C., V.L. Johnson, A. Tighe, R. Ellston, C. Haworth, T. Johnson, A. Mortlock, N. Keen, and S.S. Taylor. 2003. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. J. Cell Biol. 161:267–280.

Dossett, J.E., J.R. Bader, E.K. Trumble, K. Trimble, J.S. Breunig, E.S. Halpin, K.T. Vaughan, and E.H. Hinchcliffe. 2008. Live-cell analysis of mitotic...
spindle formation in taxol-treated cells. Cell Motil. Cytoskeleton, 65:595–613.

Ikui, A.E., C.-PH. Yang, T. Matsumoto, and S.B. Horwitz. 2005. Low concentrations of taxol cause mitotic delay followed by premature dissociation of p55CDC from Mad2 and BubR1 and abrogation of the spindle checkpoint, leading to aneuploidy. Cell Cycle, 4:1385–1388.

Jordan, M.A., D.A. Thrower, and L. Wilson. 1992. Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. J. Cell Sci. 102:401–416.

Jordan, M.A., R.J. Tosso, D.A. Thrower, and L. Wilson. 1993. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc. Natl. Acad. Sci. USA, 90:9552–9556.

Kapoor, T.M., T.U. Mayer, M.L. Coughlin, and T.J. Mitchison. 2000. Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. J. Cell Biol. 150:975–988.

Kapoor, T.M., M.A. Lampson, P. Hergert, L. Cameron, D. Cimini, E.D. Salmon, B.F. McEwen, and A. Khodjakov. 2006. Chromosomes can congress to the metaphase plate before bi-orientation. Science, 311:388–391.

Lampson, M.A., K. Renduchitala, A. Khodjakov, and T.M. Kapoor. 2004. Correcting improper chromosome-spindle attachments during cell division. Nat. Cell Biol. 6:232–237.

Lens, S.M.A., R.M.F. Wolthuis, R. Klompmaker, J. Kauw, R. Agami, T. Brummelkamp, G. Kops, and R.H. Medema. 2003. Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension. EMBO J. 22:2934–2947.

Loncarek, J., O. Kisurina-Evgenieva, T. Vainogradova, P. Hergert, S. La Terra, T.M. Kapoor, and A. Khodjakov. 2007. The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. Nature, 450:745–749.

Maekawa, T., R. Leslie, and R. Kuriyama. 1991. Identification of a minus end-specific microtubule-associated protein located at the mitotic poles in cultured mammalian cells. Eur. J. Cell Biol., 54:255–267.

Nicklas, R.B., J.C. Waters, E.D. Salmon, and S.C. Ward. 2001. Checkpoint signals in grasshopper meiosis are sensitive to microtubule attachment, but tension is still essential. J. Cell Sci. 114:4173–4183.

O’Connell, C.B., J. Loncarek, P. Hergert, A. Kourtidis, D.S. Conklin, and A. Khodjakov. 2008. The spindle assembly checkpoint is satisfied in the absence of interkinetochore tension during mitosis with unreplicated genomes. J. Cell Biol. 183:29–36.

Pinsky, B.A., C. Kung, K.M. Shokat, and S. Biggins. 2006. The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. Nat. Cell Biol. 8:78–83.

Rieder, C.L., and H. Maiato. 2004. Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. Dev. Cell. 7:637–651.

Ruchaud, S., M. Carmena, and W.C. Earnshaw. 2007. Chromosomal passengers: conducting cell division. Nat. Rev. Mol. Cell Biol. 8:798–812.

Shi, J., J.D. Orth, and T. Mitchison. 2008. Cell type variation in responses to antimitotic drugs that target microtubules and kinesin-5. Cancer Res. 68:3269–3276.

Skoufias, D.A., P.R. Andreassen, F.B. Lacroix, L. Wilson, and R.L. Margolis. 2001. Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. Proc. Natl. Acad. Sci. USA, 98:4492–4497.

Skoufias, D.A., S. DeBonis, Y. Saoudi, L. Lebeau, I. Crevel, R. Cross, R.H. Wade, D. Hackney, and F. Kozielzki. 2006. S-trityl-L-cysteine is a reversible, tight binding inhibitor of the human kinesin Eg5 that specifically blocks mitotic progression. J. Biol. Chem. 281:17559–17569.

Sudo, T., M. Nitta, H. Saya, and N.T. Ueno. 2004. Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint. Cancer Res., 64:2502–2508.

Tanaka, T.U., N. Rachidi, C. Janke, G. Pereira, M. Galova, E. Schiebel, M.J.R. Stark, and K. Nasmyth. 2002. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell, 108:317–329.

Tao, W., V.J. South, Y. Zhang, J.P. Davide, L. Farrell, N.E. Kohl, L. Sepp-Lorenzino, and R.B. Lobell. 2005. Induction of apoptosis by an inhibitor of the mitotic kinesin KSP requires both activation of the spindle assembly checkpoint and mitotic slippage. Cancer Cell, 8:49–59.

Uchida, K.S.K., K. Takagaki, K. Kumada, Y. Hirayama, T. Noda, and T. Hirota. 2009. Kinetochore stretching inactivates the spindle assembly checkpoint. J. Cell Biol. 184:383–390.

Vander, G., C.W. Cruysen, T. van Ham, M.J.M. Vromans, R.H. Medema, and S. M.A. Lens. 2007. The chromosomal passenger complex controls spindle checkpoint function independent from its role in correcting microtubule kinetochore interactions. Mol. Biol. Cell, 18:4553–4564.