Published in final edited form as:

Semin Cell Dev Biol. 2011 August ; 22(6): 559–565. doi:10.1016/j.semcdb.2011.03.007.

Correction of Microtubule-Kinetochores Attachment Errors: Mechanisms and Role in Tumor suppression

Robin M. Ricke¹ and Jan M. van Deursen^{1,2}

¹Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN 55905, USA

²Departments of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, USA

Abstract

During mitosis, cells segregate duplicated chromosomes with high fidelity in order to maintain genome stability. Proper attachment of sister kinetochores to spindle microtubules is critical for accurate chromosome segregation and is driven by complex mechanisms that promote the capture of unattached kinetochores and the resolution of erroneously attached kinetochores. Defects in these surveillance systems promote chromosome segregation and aneuploidy and can contribute to neoplastic transformation. Understanding, how, at the molecular level, accurate chromosome segregation is achieved may be crucial for our understanding of how cancer cells develop genome instability.

Keywords

Aurora B; Chromosome Passenger Complex (CPC); Mitotic Checkpoint; APC/C; Microtubule-Kinetochores Attachment

1. Introduction

During each cell cycle, replicated chromosomes must be evenly distributed into daughter cells to maintain genome stability and cellular viability. To ensure faithful segregation of chromosomes, microtubules emanating from spindle poles attach to and align chromosomes at the metaphase plate before segregating the sister chromatids evenly between daughter cells. Towards this aim, kinetochores, or macromolecular protein complexes associated with centromeres, must stably attach to dynamic microtubules. Eukaryotic cells have developed a surveillance system, the mitotic checkpoint (also referred to as the spindle assembly checkpoint), to delay anaphase onset until all chromosomes are properly attached. Kinetochores serve as the physical locale where checkpoint signaling initiates [1]. The target of the mitotic checkpoint is the APC/C (anaphase promoting complex/cyclosome), an E3 ubiquitin ligase that targets various cell cycle regulators such as Securin and Cyclin B for degradation by the 26S proteasome [2, 3]. Separase, a protease that is kept inactive through binding of Securin and phosphorylation by Cdk1/Cyclin B, then cleaves the Scc1 unit of cohesin, thereby permitting sister chromatid disjunction and anaphase onset. Thus,

© 2011 Elsevier Ltd. All rights reserved.

*Address correspondence to Jan van Deursen, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, Tel: 507-284-2524; Fax: 507-266-0340, vandeursen.jan@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

activation of the APC/C and the ensuing proteolytic degradation of mitotic proteins provide an irreversible push to cell cycle progression and division.

During metaphase, paired sister chromatids (replicated chromosomes) align at the spindle equator after sister kinetochores capture microtubules emanating from opposite spindle poles. The main goal of the mitotic checkpoint is to promote accurate chromosome separation by delaying anaphase onset until proper spindle attachment of all mitotic chromosomes has been achieved. A so-called “error correction machinery” acts to repair improper attachments. In this review we will focus on aberrant kinetochore-microtubule attachments and how the error correction machinery contributes to their resolution.

2. Detecting Aberrant Kinetochore-Microtubule Attachments

Kinetochores are said to have amphitelic attachment when sister kinetochores are bound to microtubules emanating from opposing spindle poles (Figure 1). Although the back-to-back arrangement of kinetochores itself is thought to favor bi-orientation [5, 6], various kinds of improper attachments are known to occur. Monotelic orientation occurs when one kinetochore is attached to microtubules from one pole but the sister kinetochore remains unattached. Without equivocation, unattached kinetochores are detected by the mitotic checkpoint, and thus, cells undergo a delay mediated by APC/C inhibition when encountering this type of error (Figure 1). Two forms of attachments can occur which would fail to undergo tension. Syntelic orientation occurs when both sister kinetochores are bound by microtubules emanating from the same pole and merotelic orientations occur when a sister kinetochore is bound by microtubules emanating from both spindle poles, rather than just one (Figure 1). Both merotelic and syntelic attachments are thought to be resolved by the error correction machinery, the principal component of which is Aurora B kinase. The extent to which the error correction machinery crosstalks with the mitotic checkpoint remains a hotly debated issue [4]. Much of the discussion concentrates on whether Aurora B is a bona fide mitotic checkpoint component or not. However, it should be pointed out that in addition to Aurora B, several known kinetochore-associated components of the mitotic checkpoint, such as Bub1, BubR1, CENP-E and Mps1, may contribute to chromosome-microtubule attachment [7-10], implying that cross-talk between the mitotic checkpoint and error correction machinery may extend beyond Aurora B.

3. The Attachment Error Correction Machinery

3.a. Aurora B: The Key Regulator of Attachment Error Correction

Aurora B is a highly conserved serine/threonine kinase that ensures accuracy of kinetochore-microtubule interactions during mitosis. Aurora B is the enzymatic contributor of the chromosome passenger complex (CPC), which consists of the scaffold protein INCENP as well as Survivin and Borealin [11]. Ensuring the correct bi-orientation of chromosomes on the mitotic spindle is the primary purpose of centromeric Aurora B, however, the exact mechanism for how Aurora B senses unstable attachments remains unknown. It is generally believed Aurora B corrects both syntelic and merotelically attached microtubules [12, 13]. The emerging view is that Aurora B mediated phosphorylation of key substrates acts to destabilize aberrant attachments. Once bi-orientation occurs, physical pulling stretches the kinetochores outside of the Aurora B activity zone, thereby shielding microtubules at kinetochores from the depolymerization machinery and promoting stable attachment [14]. Remodeling of the attachments between kinetochores and microtubules is thought to occur in the inner centromeric region. Consistent with this notion, Aurora B that is artificially tethered to the outer kinetochore promotes microtubule destabilization and prevents the stabilization of bi-orientated attachments [14]. However, a rather provocative report indicates that Aurora B itself may not be required for stable attachment. Biochemical work

performed with *in vitro* purified kinetochores isolated from budding yeast demonstrated that tension alone might stabilize kinetochore microtubule attachments [15]. The authors suggest that binding of microtubules to kinetochores behave in a “catch bond”, similar to a seat belt, which locks in place when pulled abruptly [15, 16]. Together, this suggests that Aurora B acts to resolve improperly attached kinetochores from microtubules but once proper attachment occurs, physical tension is sufficient to stabilize the attachment and drive the bi-orientated kinetochore out of the Aurora B activity zone.

There is accumulating evidence as to how Aurora B may act to resolve improper attachments. Phosphorylation of key Aurora B substrates may reduce the binding of microtubules to kinetochores, resulting in the unleashing of inappropriately bound microtubules [17]. The resulting “naked” kinetochore rapidly associates with another bundle of microtubules to again attempt proper attachment. Among key Aurora B substrates are kinetochore proteins known to be involved in microtubule capture. The conserved nine-subunit KMN complex (Knl1/Blinkin, Mis12, Ndc80/Hec1) forms the core of the kinetochore microtubule interface [18]. Aurora B-mediated phosphorylation of Hec1 is believed to cause a strong decrease in microtubule binding [17, 19, 20]. Hec1 phosphorylation occurs early in mitosis and decreases as cells complete bi-orientation [20]. One interpretation of these data is that phosphorylation of Hec1 early in mitosis, when kinetochores are under low tension, reduces the association of the kinetochore to microtubules to destabilize improper attachments. Dephosphorylation of Hec1 after chromosome bi-orientation might promote stable kinetochore-microtubule attachment. Furthermore, the relatively low amount of Hec1 phosphorylation in metaphase implies other substrates regulate kinetochore microtubule attachments later in mitosis.

In addition to Hec1, a Mis12 complex component Dsn1, and also Knl1, were shown to be Aurora B substrates and their phosphorylation decreases only slightly from prometaphase to metaphase.. Phosphorylation of these proteins is thought to modulate microtubule association, particularly where attachments must be corrected [21]. The current idea is that distal kinetochore associated factors, like Hec1, are dephosphorylated first following the establishment of tension and proteins closer to the inner centromere, like Knl1 and Dsn1, are dephosphorylated later [21].

In addition to KMN components, Aurora B is thought to mediate kinetochore-microtubule attachment by altering the activity of MCAK (Mitotic Centromere Associated Kinesin), which has potent microtubule-depolymerizing properties [22]. Phosphorylation of MCAK by Aurora B actually inhibits MCAK's depolymerizing activity and both Aurora B and MCAK are enriched at kinetochores with merotelic attachments [23-25]. One idea is that Aurora B mediated phosphorylation of MCAK results in enriching MCAK at aberrant attachments to promote correction, although it is currently unclear how this works.

3.b. Modulators of Aurora B function

3.b.i. INCENP—Assisting in controlling Aurora B's localization and activity levels are the associated CPC partners, INCENP, Borealin and Survivin. INCENP (INner CENTromere Protein) is a key member of the CPC that serves to regulate Aurora B catalytic activity. INCENP is highly conserved and shows the characteristic pattern of CPC localization during mitosis [26]. Defects in INCENP lead to errors in chromosome congression, chromosome segregation, cytokinesis, and spindle formation [26, 27]. Aurora B associates with INCENP through INCENP's conserved IN-box [26, 28, 29], and the resulting complex is required for proper localization of Aurora B to inner centromeric regions [26]. Aurora B also requires INCENP for localization to inner centromeric regions [26]. Aurora B and INCENP form a positive feedback loop for Aurora B enzymatic activation. The C-terminus of INCENP contains a TSS motif, which is thought to serve as a substrate for Aurora B *in trans* [29-31].

Analysis of Aurora B activity has shown that Aurora B alone has limited *in vitro* kinase activity [28-30, 32]. However, Aurora B catalysis dramatically increases when INCENP is added to the reaction and INCENP's C-terminal TSS motif becomes phosphorylated by Aurora B [28-31]. Mutation of the INCENP TSS motif decreases Aurora B activity *in vitro*, but Aurora B auto-phosphorylation is unchanged [29, 30]. Thus, while auto-phosphorylation of Aurora B at T232 has been linked to increased activity [33], auto-activated Aurora B does not represent a complete assessment of Aurora B activity (Figure 2A). These findings indicate that while Aurora B auto-activation contributes to activation, it is insufficient for maximal activation and suggest that Aurora B activity *in vivo* is stepwise.

In addition to INCENP phosphorylation altering Aurora B catalytic activity, phosphorylation of INCENP by Cdk1 may influence INCENP localization to centromeric regions [34]. INCENP has also been reported to be phosphorylated *in vitro* by Bub1 kinase [35]. Taken together, this indicates that the intricacies of INCENP function have yet to be fully worked out (see section 3.c. “working model for Aurora B activity” for further discussion).

3.b.ii. Survivin—The second CPC component is Survivin. In yeast, mutants for Aurora B (Ipl1), INCENP (Sli15) and Survivin (Bir1) phenocopy each other [36, 37]. Survivin's mitotic localization mirrors Aurora B and INCENP [38]. Whether Survivin directly influences Aurora B catalytic activity has remained controversial since two groups have reported that the addition of Survivin increases Aurora B catalytic activity *in vitro* [39, 40], while another found that Survivin did not detectably increase catalysis [31]. Survivin phosphorylation may contribute to this mechanism of Aurora B activation, although the details are unclear [39, 41]. Although Survivin was thought to contribute to CPC centromeric localization [42], the exact mechanism was unknown until recently. Three studies clearly demonstrated that Aurora B is localized to centromeric regions in a Survivin-dependent manner [43-45]. Survivin contains an evolutionary conserved BIR (Baculovirus IAP Repeat) domain, which recognizes phosphorylated threonine 3 on histone H3 [43-45]. This histone modification catalyzed by the kinase Haspin, recruits Survivin and Aurora B [43-45]. Haspin is positioned to phosphorylate histone H3 around centromeric regions due to its interaction with cohesin, which is concentrated at centromeric regions [45]. Disabling the centromeric recruitment of Aurora B through depletion of Haspin kinase only slightly modulated the catalytic activity of Aurora B, such that phosphorylation of MCAK was affected but not other known Aurora B substrates such as CENP-A and Histone H3 [43]. This implies the existence of different pools of Aurora B and is consistent with the notion of a stepwise Aurora B activation *in vivo*. Curiously, downregulation of Haspin promotes metaphase misalignment, while MCAK insufficiency has been linked to merotelic [46]. This finding supports the notion that Aurora B activity is required for error correction of both syntelic and merotelic attachments.

3.b.iii. Borealin—The third auxiliary member of the CPC is Borealin (also called DasraB). Borealin was isolated in a proteomic screen for human proteins associated with histone-depleted chromosomes [47]. Dasra-B was shown at the same time to assist in stabilizing microtubules in *Xenopus* [48]. While several studies have found that Borealin is a phosphoprotein [8, 47, 49-51], both the kinase responsible for this post-translation modification and the molecular consequences are unclear [8, 52-56]. It is important to point out that not all Aurora B is in a complex with Borealin. In fact, it has been proposed that half of the Aurora B is bound by INCENP and the other half associates with INCENP, Survivin, and Borealin together [47].

3.b.iv. TD-60—Another protein that interacts with the CPC is TD-60 (telophase disk 60 kDa). The exact function of TD-60 in the complex is unclear but TD-60's localization matches CPC members and other CPC members are delocalized in the absence of TD-60

[57]. TD-60 is required for histone H3 phosphorylation at threonine 3 [58], a modification by Haspin that recruits Survivin and Aurora B. Interestingly, although TD-60 does not co-precipitate with Borealin-containing complexes [47], it does interact with some Aurora B complexes containing INCENP [58]. The addition of TD-60 with microtubules promotes activation of Aurora B kinase *in vitro* [58], indicating that in certain situations, TD-60 could affect Aurora B activity.

3.c. Working Model for Aurora B Activation

There is consensus that inner centromere-associated Aurora B plays a central role in the attachment error correction machinery, but many key questions remain. For instance, biochemical studies suggest that Aurora B resides in multiple different protein complexes. Specifically, Borealin fails to co-precipitate TD-60, but both proteins precipitate with Aurora B [47, 58] (Figure 2B). Moreover, Aurora B associates with INCENP independent of Borealin, Survivin and TD-60 [47] (Figure 2B). One idea would be that each of the Aurora B subcomplexes occupies a distinct subcellular niche or preferentially targets certain substrates, or both. The recent finding that depletion of Haspin only affects phosphorylation of select Aurora B substrates supports this model [43].

Post-translational modifications of Aurora B modulators might transform the activation status of certain Aurora B subcomplexes as phosphorylated Borealin, Survivin, and INCENP are associated with increased Aurora B enzymatic activity *in vitro* [8, 29-31, 41] (Figure 2B). Other *in vitro* studies suggest that Bub1 and Cdk1 may also modulate Aurora B activity through INCENP [34, 35]. Furthermore, Mst1 kinase has been shown to restrict Aurora B autophosphorylation, implying the need for a non-phosphorylated Aurora B pool in mitosis [59]. Besides phosphorylation, Aurora B is also subject to sumoylation [60]. Thus, a major future challenge will be to understand the various post-translational modifications of Aurora B complexes and their functional implications.

Aurora B and INCENP turnover is high at inner centromeric regions [61]. This raises the possibility that active Aurora B complexes are generated at inner centromeres from where they may diffuse to different locations, including the outer kinetochore, which contains substrates such as Hec1, Knl1, Dsn1 [20, 21, 62]. The impact of kinetochore-microtubule attachments on the composition and activity of Aurora B complexes at inner centromeres has not been established (Figure 2B). It would also be important to determine whether Aurora B and INCENP turnover at inner centromeres is dependent on attachment status. Given that the activity of kinetochore-associated mitotic checkpoint proteins alters upon kinetochore-microtubule attachment and given that some of these proteins have been implicated in regulating Aurora B activity, it is reasonable to assume that the composition and/or turnover of Aurora B complexes at inner centromeres is influenced by attachment status. Based on the above considerations, we conclude that the *in vivo* activation of Aurora B is likely incredibly complex.

4. Potential mechanisms through which merotelic attachments may promote tumorigenesis

4.a. Centrosome Amplification

Cancer cells often contain increased numbers of centrosomes, but how this affects the fidelity of chromosome segregation, until recently, remained rather enigmatic. Ganem et al. observed that cultured cells with multiple centrosomes routinely undergo bipolar cell divisions, but with an increased frequency of lagging chromosomes in anaphase [63]. Additionally, Silkworth et al. observed an increase in merotelic attachments in multipolar colorectal cancer cells compared to bipolar cells [64]. The model proposed is that lagging

chromosomes in cells with centrosome amplification occur as a consequence passing through a transient, multipolar intermediate where kinetochores are attached to differing spindle poles. If the two spindle poles with the same chromosome attached were to cluster, then both kinetochores would be pulled to the same daughter cell in a syntelic manner. Merotelically occurs if the two spindles cluster in opposing manners. Although the evidence presented from both groups is quite supportive of this model, there has yet to be live-cell imaging data that unequivocally demonstrate a multipolar intermediate that resolves with a lagging chromosome. It has been suggested that merotelic attachments persist because the error correction machinery could be inefficient and overwhelmed in tumor cells [65]. However, increased centriole number also correlates with increased anaphase lagging chromosomes in primary cells [66]. Additionally, the contribution of syntelic attachments to near-diploid aneuploidy in these cells is unclear. One would predict the frequencies of syntely and merotelically to be quite similar. The observation that these cells have lagging chromosomes but not metaphase misalignments suggests that syntelic attachments might be corrected. Given that Aurora B mediates the resolution of both syntelic and merotelic attachments, it is not clear why merotelically remains. Finally, not all tumors have extranumerary centrosomes and cancer lines with two centrosomes often exhibit elevated rates of lagging chromosomes [63], indicating that this is not a universal mechanism for lagging chromosomes.

4.b. Overexpression of Aurora B

Aurora B protein is overexpressed in many human tumors and was identified as a biomarker for tumors exhibiting chromosomal instability [67, 68]. Whether Aurora B overexpression is oncogenic or merely reflective of the increased proliferative index of a tumor is not clear. A mouse model that mimics the ubiquitous overexpression of Aurora B will be necessary to clarify this issue. Several groups have attempted to uncover the molecular mechanism for how Aurora B overexpression may deregulate chromosome segregation. This analysis is complicated by the observation that extreme overexpression results in Aurora B mislocalization whereas mild Aurora B overexpression (3-5 fold) correctly targets to inner centromeric regions [69, 70]. Based on this, the molecular consequences of Aurora B overexpression may depend on the fold change of Aurora B expression. Indeed, two different cellular phenotypes have been observed with Aurora B overexpression, an increase in tetraploidy and an increase in near-diploid aneuploidy [71-73]. Whether near-diploid aneuploidy is a consequence of mild overexpression and tetraploidy results from high overexpression remains to be seen. The exact molecular mechanism of how Aurora B overexpression may disrupt cell cycle progression is not known as overexpression could increase overall Aurora B activity or alternatively, could result in reduced activity if Aurora B were to titrate some other required factor(s) away from the inner centromeric zone.

4.b. Deregulation of Aurora B modulators

Another mechanism that may disrupt normal Aurora B catalysis and prevent proper error correction would be the dysregulation of Aurora B modulators. This could occur by altered protein expression. Both overexpression of Borealin and Survivin has been noted in several human tumors, although increases in INCENP have not been described (<http://www.oncomine.org>). As with Aurora B overexpression, whether overexpression of these CPC proteins is oncogenic is not known. Because Survivin is a bi-functional protein that is also involved in signaling apoptosis, the exact mechanism by which Survivin may promote tumorigenesis is unclear [74]. Overexpression of Survivin has been linked with numerous kinds of human tumors including breast, lung, liver, sarcomas, lymphomas and colon [75]. Alternatively, altering the post-translational modifications of Aurora B modulators might also change substrate specificity or total activity of Aurora B. Whether altered post-translational modifications contribute to tumorigenesis will remain unclear until

the kinases responsible for phosphorylating various CPC members are firmly established. One intriguing report suggested that Mst1 limits Aurora B activity [59]. Cells depleted for Mst1 demonstrated chromosome congression errors and increased Aurora B auto-phosphorylation [59]. This suggests that the cell requires an optimal amount of Aurora B activity and suggests that both hyper- and hypo- activation of Aurora B is detrimental. Finally, overexpression of the E3 SUMO ligase for Aurora B may promote its premature de-activation, although the E3 SUMO ligase responsible is currently not known. Given the multiple layers of regulation on Aurora B activity during mitotic progression, there seem to be many ways for Aurora B deregulation.

5. Conclusions

Most cancer cells seem to have a functional mitotic checkpoint [76], which suggests that mitotic checkpoint defects may not represent a widespread mechanism in the acquisition of whole chromosome instability (W-CIN) in tumors. Despite exhaustive attempts, mutations in W-CIN genes are rare [77-79]. Given the high degree of genetic alterations, the proliferative properties of a tumor may not represent the cellular precursors and unstable intermediates that formed that tumor. This seems especially true for genes that contribute to W-CIN since tumors in at least one murine model were not addicted to the initiating oncogene, Mad2 [80]. On the other hand, epigenetic alterations of checkpoint genes seem more frequently targeted in tumors [67], mostly promoting upregulation, the consequences of which have only been assessed for a few genes in mouse [66, 81, 82]. We are excited about the potential to model defects that might result in merotelic attachment in mice and determine whether spontaneous tumorigenesis results. Further work is necessary to exactly pinpoint the alterations that may drive W-CIN during tumor derivation, particularly for merotelic attachment defects.

Acknowledgments

J.V.D is supported by the National Institutes of Health (CA96985, CA77262 and CA126828). R.M.R. is the recipient of a Leukemia and Lymphoma Society fellowship. We thank Drs. Paul Galardy and Liviu Malureanu for critical reading of this manuscript.

References

1. Gascoigne KE, Cheeseman IM. Kinetochore assembly: if you build it, they will come. *Curr Opin Cell Biol.* 2010
2. Peters JM. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol.* 2006; 7:644–56. [PubMed: 16896351]
3. Pines J. Mitosis: a matter of getting rid of the right protein at the right time. *Trends Cell Biol.* 2006; 16:55–63. [PubMed: 16337124]
4. Nezi L, Musacchio A. Sister chromatid tension and the spindle assembly checkpoint. *Curr Opin Cell Biol.* 2009; 21:785–95. [PubMed: 19846287]
5. Indjeian VB, Murray AW. Budding yeast mitotic chromosomes have an intrinsic bias to biorient on the spindle. *Curr Biol.* 2007; 17:1837–46. [PubMed: 17980598]
6. Loncarek J, Kisurina-Evgenieva O, Vinogradova T, Hergert P, La Terra S, Kapoor TM, et al. The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. *Nature.* 2007; 450:745–9. [PubMed: 18046416]
7. Meraldi P, Sorger PK. A dual role for Bub1 in the spindle checkpoint and chromosome congression. *Embo J.* 2005; 24:1621–33. [PubMed: 15933723]
8. Jelluma N, Brenkman AB, van den Broek NJ, Cruijisen CW, van Osch MH, Lens SM, et al. Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment. *Cell.* 2008; 132:233–46. [PubMed: 18243099]

9. Elowe S, Dulla K, Uldschmid A, Li X, Dou Z, Nigg EA. Uncoupling of the spindle-checkpoint and chromosome-congression functions of BubR1. *J Cell Sci.* 2010; 123:84–94. [PubMed: 20016069]
10. Kim Y, Holland AJ, Lan W, Cleveland DW. Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of CENP-E. *Cell.* 2010; 142:444–55. [PubMed: 20691903]
11. Ruchaud S, Carmenta M, Earnshaw WC. Chromosomal passengers: conducting cell division. *Nat Rev Mol Cell Biol.* 2007; 8:798–812. [PubMed: 17848966]
12. Tanaka TU, Rachidi N, Janke C, Pereira G, Galova M, Schiebel E, et al. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell.* 2002; 108:317–29. [PubMed: 11853667]
13. Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, et al. The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J Cell Biol.* 2003; 161:281–94. [PubMed: 12707311]
14. Liu D, Vader G, Vromans MJ, Lampson MA, Lens SM. Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. *Science.* 2009; 323:1350–3. [PubMed: 19150808]
15. Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-Santoyo H, et al. Tension directly stabilizes reconstituted kinetochore-microtubule attachments. *Nature.* 2010; 468:576–9. [PubMed: 21107429]
16. Shimamoto Y, Kapoor TM. Mechanoregulation: Cellular seat belts. *Nature.* 2010; 468:518–9. [PubMed: 21107420]
17. Cheeseman IM, Anderson S, Jwa M, Green EM, Kang J, Yates JR 3rd, et al. Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell.* 2002; 111:163–72. [PubMed: 12408861]
18. Wan X, O'Quinn RP, Pierce HL, Joglekar AP, Gall WE, DeLuca JG, et al. Protein architecture of the human kinetochore microtubule attachment site. *Cell.* 2009; 137:672–84. [PubMed: 19450515]
19. DeLuca JG, Gall WE, Ciferri C, Cimini D, Musacchio A, Salmon ED. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. *Cell.* 2006; 127:969–82. [PubMed: 17129782]
20. Deluca KF, Lens SM, Deluca JG. Temporal changes in Hec1 phosphorylation control kinetochore-microtubule attachment stability during mitosis. *J Cell Sci.* 2011; 124:622–34. [PubMed: 21266467]
21. Welburn JP, Vleugel M, Liu D, Yates JR 3rd, Lampson MA, Fukagawa T, et al. Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. *Mol Cell.* 2010; 38:383–92. [PubMed: 20471944]
22. Helenius J, Brouhard G, Kalaidzidis Y, Diez S, Howard J. The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. *Nature.* 2006; 441:115–9. [PubMed: 16672973]
23. Andrews PD, Ovechkina Y, Morrice N, Wagenbach M, Duncan K, Wordeman L, et al. Aurora B regulates MCAK at the mitotic centromere. *Dev Cell.* 2004; 6:253–68. [PubMed: 14960279]
24. Lan W, Zhang X, Kline-Smith SL, Rosasco SE, Barrett-Wilt GA, Shabanowitz J, et al. Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. *Curr Biol.* 2004; 14:273–86. [PubMed: 14972678]
25. Knowlton AL, Lan W, Stukenberg PT. Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. *Curr Biol.* 2006; 16:1705–10. [PubMed: 16950107]
26. Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmenta M, Smythe C, et al. INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr Biol.* 2000; 10:1075–8. [PubMed: 10996078]
27. Mackay AM, Ainsztein AM, Eckley DM, Earnshaw WC. A dominant mutant of inner centromere protein (INCENP), a chromosomal protein, disrupts prometaphase congression and cytokinesis. *J Cell Biol.* 1998; 140:991–1002. [PubMed: 9490714]
28. Kang J, Cheeseman IM, Kallstrom G, Velmurugan S, Barnes G, Chan CS. Functional cooperation of Dam1, Ipl1, and the inner centromere protein (INCENP)-related protein Sli15 during chromosome segregation. *J Cell Biol.* 2001; 155:763–74. [PubMed: 11724818]

29. Sessa F, Mapelli M, Ciferri C, Tarricone C, Areces LB, Schneider TR, et al. Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. *Mol Cell*. 2005; 18:379–91. [PubMed: 15866179]
30. Bishop JD, Schumacher JM. Phosphorylation of the carboxyl terminus of inner centromere protein (INCENP) by the Aurora B Kinase stimulates Aurora B kinase activity. *J Biol Chem*. 2002; 277:27577–80. [PubMed: 12048181]
31. Honda R, Korner R, Nigg EA. Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol Biol Cell*. 2003; 14:3325–41. [PubMed: 12925766]
32. Biggins S, Severin FF, Bhalla N, Sassoon I, Hyman AA, Murray AW. The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev*. 1999; 13:532–44. [PubMed: 10072382]
33. Yasui Y, Urano T, Kawajiri A, Nagata K, Tatsuka M, Saya H, et al. Autophosphorylation of a newly identified site of Aurora-B is indispensable for cytokinesis. *J Biol Chem*. 2004; 279:12997–3003. [PubMed: 14722118]
34. Goto H, Kiyono T, Tomono Y, Kawajiri A, Urano T, Furukawa K, et al. Complex formation of Plk1 and INCENP required for metaphase-anaphase transition. *Nat Cell Biol*. 2006; 8:180–7. [PubMed: 16378098]
35. Boyarchuk Y, Salic A, Dasso M, Arnaoutov A. Bub1 is essential for assembly of the functional inner centromere. *J Cell Biol*. 2007; 176:919–28. [PubMed: 17389228]
36. Uren AG, Beilharz T, O'Connell MJ, Bugg SJ, van Driel R, Vaux DL, et al. Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division. *Proc Natl Acad Sci U S A*. 1999; 96:10170–5. [PubMed: 10468581]
37. Kim JH, Kang JS, Chan CS. Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerevisiae*. *J Cell Biol*. 1999; 145:1381–94. [PubMed: 10385519]
38. Skoufias DA, Mollinari C, Lacroix FB, Margolis RL. Human survivin is a kinetochore-associated passenger protein. *J Cell Biol*. 2000; 151:1575–82. [PubMed: 11134084]
39. Bolton MA, Lan W, Powers SE, McClelland ML, Kuang J, Stukenberg PT. Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation. *Mol Biol Cell*. 2002; 13:3064–77. [PubMed: 12221116]
40. Chen J, Jin S, Tahir SK, Zhang H, Liu X, Sarthy AV, et al. Survivin enhances Aurora-B kinase activity and localizes Aurora-B in human cells. *J Biol Chem*. 2003; 278:486–90. [PubMed: 12419797]
41. Chu Y, Yao PY, Wang W, Wang D, Wang Z, Zhang L, et al. Aurora B kinase activation requires survivin priming phosphorylation by PLK1. *J Mol Cell Biol*. 2010
42. Vader G, Kauw JJ, Medema RH, Lens SM. Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. *EMBO Rep*. 2006; 7:85–92. [PubMed: 16239925]
43. Wang F, Dai J, Daum JR, Niedzialkowska E, Banerjee B, Stukenberg PT, et al. Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. *Science*. 2010; 330:231–5. [PubMed: 20705812]
44. Kelly AE, Ghenoiu C, Xue JZ, Zierhut C, Kimura H, Funabiki H. Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B. *Science*. 2010; 330:235–9. [PubMed: 20705815]
45. Yamagishi Y, Honda T, Tanno Y, Watanabe Y. Two histone marks establish the inner centromere and chromosome bi-orientation. *Science*. 2010; 330:239–43. [PubMed: 20929775]
46. Dai J, Sultan S, Taylor SS, Higgins JM. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes Dev*. 2005; 19:472–88. [PubMed: 15681610]
47. Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, et al. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J Cell Biol*. 2004; 166:179–91. [PubMed: 15249581]

48. Sampath SC, Ohi R, Leismann O, Salic A, Pozniakovski A, Funabiki H. The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. *Cell*. 2004; 118:187–202. [PubMed: 15260989]
49. Nousiainen M, Sillje HH, Sauer G, Nigg EA, Korner R. Phosphoproteome analysis of the human mitotic spindle. *Proc Natl Acad Sci U S A*. 2006; 103:5391–6. [PubMed: 16565220]
50. Hayama S, Daigo Y, Yamabuki T, Hirata D, Kato T, Miyamoto M, et al. Phosphorylation and activation of cell division cycle associated 8 by aurora kinase B plays a significant role in human lung carcinogenesis. *Cancer Res*. 2007; 67:4113–22. [PubMed: 17483322]
51. Tsukahara T, Tanno Y, Watanabe Y. Phosphorylation of the CPC by Cdk1 promotes chromosome bi-orientation. *Nature*. 2010; 467:719–23. [PubMed: 20739936]
52. Sliedrecht T, Zhang C, Shokat KM, Kops GJ. Chemical genetic inhibition of Mps1 in stable human cell lines reveals novel aspects of Mps1 function in mitosis. *PLoS One*. 2010; 5:e10251. [PubMed: 20422024]
53. Maciejowski J, George KA, Terret ME, Zhang C, Shokat KM, Jallepalli PV. Mps1 directs the assembly of Cdc20 inhibitory complexes during interphase and mitosis to control M phase timing and spindle checkpoint signaling. *J Cell Biol*. 2010; 190:89–100. [PubMed: 20624902]
54. Santaguida S, Tighe A, D'Alise AM, Taylor SS, Musacchio A. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J Cell Biol*. 2010; 190:73–87. [PubMed: 20624901]
55. Hewitt L, Tighe A, Santaguida S, White AM, Jones CD, Musacchio A, et al. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. *J Cell Biol*. 2010; 190:25–34. [PubMed: 20624899]
56. Maure JF, Kitamura E, Tanaka TU. Mps1 kinase promotes sister-kinetochore bi-orientation by a tension-dependent mechanism. *Curr Biol*. 2007; 17:2175–82. [PubMed: 18060784]
57. Andreassen PR, Palmer DK, Wener MH, Margolis RL. Telophase disc: a new mammalian mitotic organelle that bisects telophase cells with a possible function in cytokinesis. *J Cell Sci*. 1991; 99(Pt 3):523–34. [PubMed: 1939370]
58. Rosasco-Nitcher SE, Lan W, Khorasanizadeh S, Stukenberg PT. Centromeric Aurora-B activation requires TD-60, microtubules, and substrate priming phosphorylation. *Science*. 2008; 319:469–72. [PubMed: 18218899]
59. Oh HJ, Kim MJ, Song SJ, Kim T, Lee D, Kwon SH, et al. MST1 limits the kinase activity of aurora B to promote stable kinetochore-microtubule attachment. *Curr Biol*. 2010; 20:416–22. [PubMed: 20171103]
60. Fernandez-Miranda G, Perez de Castro I, Carmena M, Aguirre-Portoles C, Ruchaud S, Fant X, et al. SUMOylation modulates the function of Aurora-B kinase. *J Cell Sci*. 2010; 123:2823–33. [PubMed: 20663916]
61. Ahonen LJ, Kukkonen AM, Pouwels J, Bolton MA, Jingle CD, Stukenberg PT, et al. Perturbation of Incenp function impedes anaphase chromatid movements and chromosomal passenger protein flux at centromeres. *Chromosoma*. 2009; 118:71–84. [PubMed: 18784935]
62. Lampson MA, Cheeseman IM. Sensing centromere tension: Aurora B and the regulation of kinetochore function. *Trends Cell Biol*. 2010
63. Ganem NJ, Godinho SA, Pellman D. A mechanism linking extra centrosomes to chromosomal instability. *Nature*. 2009; 460:278–82. [PubMed: 19506557]
64. Silkworth WT, Nardi IK, Scholl LM, Cimini D. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PLoS One*. 2009; 4:e6564. [PubMed: 19668340]
65. Bakhoun SF, Compton DA. Cancer: CINful centrosomes. *Curr Biol*. 2009; 19:R642–5. [PubMed: 19674544]
66. van Ree JH, Jeganathan KB, Malureanu L, van Deursen JM. Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. *J Cell Biol*. 2010; 188:83–100. [PubMed: 20065091]
67. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet*. 2006; 38:1043–8. [PubMed: 16921376]

68. Lin ZZ, Jeng YM, Hu FC, Pan HW, Tsao HW, Lai PL, et al. Significance of Aurora B overexpression in hepatocellular carcinoma. *Aurora B Overexpression in HCC. BMC Cancer.* 2010; 10:461. [PubMed: 20799978]
69. Ditchfield C, Johnson VL, Tighe A, Ellston R, Haworth C, Johnson T, et al. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J Cell Biol.* 2003; 161:267–80. [PubMed: 12719470]
70. Girdler F, Gascoigne KE, Eysers PA, Hartmuth S, Crafter C, Foote KM, et al. Validating Aurora B as an anti-cancer drug target. *J Cell Sci.* 2006; 119:3664–75. [PubMed: 16912073]
71. Tatsuka M, Katayama H, Ota T, Tanaka T, Odashima S, Suzuki F, et al. Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. *Cancer Res.* 1998; 58:4811–6. [PubMed: 9809983]
72. Ota T, Suto S, Katayama H, Han ZB, Suzuki F, Maeda M, et al. Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Res.* 2002; 62:5168–77. [PubMed: 12234980]
73. Nguyen HG, Makitalo M, Yang D, Chinnappan D, St Hilaire C, Ravid K. Deregulated Aurora-B induced tetraploidy promotes tumorigenesis. *FASEB J.* 2009; 23:2741–8. [PubMed: 19332642]
74. Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer.* 2003; 3:46–54. [PubMed: 12509766]
75. Carrasco RA, Stamm NB, Marcusson EG, Sandusky GE, Iversen P, Patel BK. Antisense Inhibition of Survivin Expression as a Cancer Therapeutic. *Mol Cancer Ther.* 2011
76. Tighe A, Johnson VL, Albertella M, Taylor SS. Aneuploid colon cancer cells have a robust spindle checkpoint. *EMBO Rep.* 2001; 2:609–14. [PubMed: 11454737]
77. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science.* 2006; 314:268–74. [PubMed: 16959974]
78. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. *Nature.* 2007; 446:153–8. [PubMed: 17344846]
79. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. *Science.* 2007; 318:1108–13. [PubMed: 17932254]
80. Sotillo R, Schvartzman JM, Socci ND, Benezra R. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. *Nature.* 2010; 464:436–40. [PubMed: 20173739]
81. Sotillo R, Hernando E, Diaz-Rodriguez E, Teruya-Feldstein J, Cordon-Cardo C, Lowe SW, et al. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. *Cancer cell.* 2007; 11:9–23. [PubMed: 17189715]
82. Diaz-Rodriguez E, Sotillo R, Schvartzman JM, Benezra R. Hec1 overexpression hyperactivates the mitotic checkpoint and induces tumor formation in vivo. *Proc Natl Acad Sci U S A.* 2008; 105:16719–24. [PubMed: 18940925]

Figure 1. Kinetochore Microtubules Attachment Errors

Proper chromosome segregation is achieved by amphitelic attachment. Kinetochores with unattached or monotelic attachment elicit a mitotic checkpoint signal. Syntelic and merotelically attached chromosomes fail to undergo tension and their kinetochores remain within the zone of Aurora B activity.

Figure 2. Regulation of Aurora B Activity

A) Auto-activation of Aurora B is not an indicator for maximal Aurora B activation.
 B) Different subcomplexes of Aurora B have been isolated biochemically and each subcomplex may target substrates differentially. Since both Aurora B and INCENP turnover rapidly at the inner centromere, it is not known whether these subcomplexes impact centromere-, kinetochore, or microtubule-associated substrates.