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Correcting aberrant kinetochore microtubule attachments: a hidden regulation of Aurora B on microtubules

Hironori Funabiki¹

¹Laboratory of Chromosome and Cell biology, The Rockefeller University. funabih@rockefeller.edu

Abstract

For equal chromosome segregation, a pair of kinetochores on each duplicated chromosome must attach to microtubules connecting to opposite poles. The protein kinase Aurora B plays a critical role in destabilizing microtubules attached in a wrong orientation through phosphorylating kinetochore proteins. The mechanism behind this selective destabilization of aberrant attachments remains elusive. While Aurora B is most enriched on the centromere from prophase to metaphase, emerging evidence suggests the importance of Aurora B on microtubules in this process. Here I discuss two hypothetical models that explain the requirement of Aurora B on microtubules for selective destabilization of aberrant attachments; microtubule-induced substrate masking and treadmill-removal of Aurora B on microtubules proximal to polymerizing ends.

Introduction

To segregate multiple chromosomes equally to dividing cells, a bipolar spindle guides directional chromosome movements. At the centromere of each chromosome, the kinetochore establishes a load-bearing attachment to microtubules. Each kinetochore must capture plus ends of microtubules that are connected to one spindle pole, while its sister kinetochore must attach to microtubules linked to the other spindle pole (Fig.1). This configuration (amphitelic attachment) ensures equal distribution of sister chromatids upon their separation in anaphase. If both sister kinetochores attach to microtubules from the same pole (syntelic attachment), or one kinetochore attaches to microtubules from multiple poles (merotelic attachment), these aberrant attachments must be corrected before anaphase to prevent aneuploidy and formation of micronuclei. One of the key regulators of this error correction mechanism is the protein kinase Aurora B, which is essential for destabilizing erroneous attachments. Since Aurora B is most enriched on the centromere when error correction occurs in mitosis, models have proposed that the tension-dependent change in kinetochore substrate accessibility by Aurora B at the centromere dictates the detachment probability [1–3]. Here I review accumulating lines of evidence supporting the critical role of microtubule-mediated Aurora B regulation, and speculate on its contribution to the selective destabilization of aberrant microtubule attachments.

Address: Laboratory of Chromosome and Cell biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

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Basic mechanisms of correcting aberrant kinetochore-microtubule attachments

A mathematical model has indicated that geometric constraint of kinetochores and dynamic microtubule attachment can constitute a basic mechanism by which erroneous attachment is spontaneously corrected within finite time [4] (Fig. 2A, B). The geometric constraint, which limits attachment of microtubules preferentially to their proximal kinetochore, is sufficient to effectively accomplish bioriented attachment (Fig. 2A), while stochastic microtubule exchange can spontaneously displace aberrantly attached microtubules (Fig. 2B). However, this basic model may not readily explain kinetochore attachment control in meiosis I, where pairs of kinetochores on homologous chromosomes are not under apparent geometric constraint [5] (Fig. 2A). A prevailing model suggests that tension-dependent stabilization of kinetochore-microtubule attachment further contributes to the error correction mechanism [2,3].

The tension model was initially substantiated by microneedle manipulation of chromosomes in grasshopper spermatocytes, showing that tension can stabilize kinetochore microtubule attachment [6]. Biophysical experiments with purified budding yeast kinetochores further demonstrated that kinetochores attached to depolymerizing ends are much more vulnerable to detachment than those attached to polymerizing ends are, but tension can stabilize kinetochore microtubule attachments to depolymerizing ends without ATP [7–9]. Therefore, the kinetochore has an intrinsic catch bond-like capacity to change its microtubule binding property depending on tension (Fig. 2C). However, in vivo, tension-dependent error correction depends on additional enzymatic reactions, whose major player is the protein kinase Aurora B.

Microtubule detachment by Aurora B-dependent phosphorylation of kinetochore substrates

Aurora B, which forms the Chromosomal Passenger Complex (CPC) with INCENP, Borealin and Survivin (Fig.3), regulates a number of mitotic processes [3,10]. Upon mitotic entry, the CPC becomes enriched on the centromere between two pairs of sister kinetochores. Centromeric enrichment of the CPC depends on two kinds of histone phosphorylation marks. Survivin directly binds to histone H3 phosphorylated at Thr3 (H3T3ph), while Borealin binds to Sgo1, which is recruited to H2A phosphorylated at Thr120. At the metaphase-to-anaphase transition, the CPC dissociates from the centromere and relocates to the spindle midzone.

Aurora B dependent phosphorylation of the Ndc80 (Hec1 in human) subunit of the Ndc80 complex, which is critical for supporting the load-bearing microtubule attachment, facilitates microtubule detachment [9,11,12]. In human cells, the N-terminal tail of Hec1, which contributes to microtubule binding together with the adjacent calponin homology domain, is phosphorylated at nine residues by Aurora B, and microtubule binding affinity gradually decreases as the number of phosphorylation sites increases [11]. In budding yeast, multisite phosphorylation of the Dam1 complex also plays an important role in microtubule

detachment [9]. The phosphorylation status of Ndc80 and several other kinetochore proteins is sensitive to microtubule-mediated tension applied to the kinetochore [13–15]. A Förster Resonance Energy Transfer (FRET) based intramolecular tension-sensor established that the force applied at the kinetochore is generated by microtubule dynamics [16]. An intermolecular FRET based-measurement of Hec1-microtubule binding at the kinetochore in human cells visualized tension-induced microtubule binding, which is sensitive to Aurora B-dependent phosphorylation of Hec1 [17**].

The detachment probability is controlled by tension and Aurora B-dependent phosphorylation of kinetochore substrates. However, tension and complete dephosphorylation are not necessarily required for microtubule attachment. Even when tension is reduced by taxol, which stabilizes microtubule end dynamics, the majority of kinetochores maintain microtubule attachment, while Aurora B continuously generates a small number of unattached kinetochores, which activate the spindle checkpoint [18]. In cells expressing Hec1 with mutations mimicking a hyper-phosphorylated form (Hec1–9D), kinetochores can maintain attachment to depolymerizing ends [19*], although kinetochore microtubules become unstable and activate the checkpoint [20]. While Hec1 generally becomes dephosphorylated on metaphase kinetochores [13], phosphorylation of Hec1 at Ser69 is maintained and is important for suppressing formation of lagging chromosomes in anaphase, likely through destabilizing merotelic attachment [21*]. During prometaphase of mouse oocyte meiosis I, kinetochore phosphorylation levels remain high during prometaphase even on aligned chromosomes that are under full tension [22]. This kinetochore phosphorylation enables correction of merotelic attachments, which are frequently observed in female acentrosomal meiosis. Therefore, certain levels of kinetochore phosphorylation do not interfere with amphitelic attachment but rather help resolving merotelic attachment.

Once amphitelic attachment is accomplished, kinetochores must be dephosphorylated below a threshold to avoid stochastic microtubule detachment. Inhibiting Hec1 N-terminal phosphorylation (by Hec1–9A) stabilizes microtubule attachment regardless of the tension status and silences the spindle checkpoint even in the presence of unaligned chromosomes [23,24]. Therefore, in human cells, Hec1 dephosphorylation appears to be the key trigger for microtubule attachment stabilization, although the Dam1 dephosphorylation is more important in budding yeast [25]. This final microtubule stabilization, also helped by recruitment of the Ska complex (a plausible metazoan ortholog of the Dam 1 complex) and PP1 [26*,27*], is important for silencing the spindle checkpoint. The key question is how Aurora B-dependent phosphorylation is suppressed upon establishing amphitelic attachment.

Function of Aurora B on the centromere

Aurora B is activated by autophosphorylation of the activation loop and the C-terminal IN-box module of INCENP [28], and thus clustering of the CPC, such as by binding H3T3ph nucleosomes, can facilitate Aurora B activation [29–31]. Therefore, it has been speculated that the level of kinetochore phosphorylation can be regulated by the substrate accessibility of Aurora B activated on centromeric chromatin; as microtubules pull kinetochore substrates

away from the centromeric Aurora B, phosphatases on the kinetochore win over the kinase to promote dephosphorylation [1–3].

However, recent observations question this model. First, the centromere-targeting module of the CPC is dispensable for efficient bipolar kinetochore attachment and viability in budding yeast [32]. Second, in human cells, it was shown that the critical function of the centromere-targeting module of the CPC is to maintain sister chromatid cohesion specifically at the centromere through recruiting Sgo1-PP2A [33**]. Interestingly, artificial targeting of INCENP to the kinetochore can result in chromosome misalignment with unstable kinetochore attachments, but this defect can be rescued by augmenting centromeric cohesion in the absence of the CPC on the centromere [33**]. The study suggests that the major function of the CPC at the centromere is to protect sister chromatid cohesion and provide physical connection between sister kinetochores. This ensures that the force acts on one kinetochore can be transmitted to its sister kinetochore, and/or that the geometrical constraint of kinetochores can be maintained. A similar role of the CPC at the centromere is reported in budding yeast, though this functionality is not essential for viability [32].

Regulation and importance of Aurora B on microtubules

Before anaphase, Cdk1 phosphorylates Thr59 of human INCENP, inhibiting interaction with a kinesin-like motor MKLP2, which targets the CPC to microtubules at the anaphase spindle midzone [34]. In addition, the single alpha helix (SAH) domain and its flanking phosphoregulatory (PR) domain of INCENP mediate microtubule association, while Cdk1-dependent phosphorylation of PR suppresses microtubule binding [35**–41**]. However, despite the CDK1-dependent suppression of INCENP-microtubule interaction, this interaction plays important roles even before anaphase. In *Xenopus* egg extracts, the microtubule-binding capacity of the SAH domain is essential for spindle assembly [36]. In HeLa cells, the SAH domain is needed for effective phosphorylation of Hec1, and for maintaining the spindle checkpoint in cells treated with taxol [35**]. Importantly, requirement for the SAH can be bypassed by replacement with the microtubule-binding domain of MAP4, but not with a dimerization module, which activates Aurora B without any localization [35**]. Furthermore, inhibiting Cdk1-dependent phosphorylation at the PR domain of INCENP interferes with checkpoint silencing [35**]. In budding yeast, an artificially generated minimal CPC, which is composed of Ipl1 (Aurora B) and a truncated, dimerized version of Sli15 (INCENP) just containing the SAH domain and the Aurora B-interaction domain, can support bioriented microtubule attachment [41**]. Other microtubule-binding proteins, such as the Ska complex, UBASH3B and EB1 also contribute to microtubule-dependent control of Aurora B activity during pre-anaphase [42–44].

Substrate masking and treadmill-removal models of suppressing Aurora B-dependent phosphorylation

How can Aurora B on microtubules be a critical component of kinetochore phosphorylation and contribute to the tension-sensing mechanism? Here I postulate two models; substrate masking, and a treadmill-removal of Aurora B (Figure 4).

Substrate masking.

On an unattached kinetochore (Fig.4A), Aurora B does not have to bind microtubules to phosphorylate the kinetochore substrates [13,14]. However, when kinetochores are attached to microtubules (Fig. 4B, C), I speculate that CPC-binding to microtubule becomes necessary. This may be due to the fact that key microtubule binding proteins, such as Ndc80, use positively charged residues to both interact with the acidic tubulin tails [45–47], and to be recognized by the basophilic kinase Aurora B [48]. Therefore, microtubule binding, which is stable enough to support load-bearing attachment, may mask Aurora B targeting positive charges. This process is corroborated by PP2A (and PP1) at the kinetochore, which counteracts Aurora B through dephosphorylating substrates [49]. As phosphatases recognize phosphorylated substrates, which consequentially dissociate from microtubules, microtubule binding would selectively affect recognition by Aurora B but not by phosphatases, shifting a balance toward dephosphorylation. Alternatively, microtubules may apply an indirect mechanism to mask substrates. For example, the Ndc80 complex can cluster upon binding to microtubules, and a segment of the N-terminal tail of Ndc80/Hec1 becomes occluded upon oligomerization [46]. Microtubule targeting of the CPC would facilitate substrate recognition to overcome these burdens [50].

Treadmill removal.

Aurora B can be activated by microtubules [29,36,39,51], but during prometaphase, high Cdk1 activity suppresses microtubule-binding of the CPC [34,35**]. However, a small fraction of the CPC can associate with microtubules where it can eventually become activated (Fig. 4B, C)[29,36,42,44]. At a microtubule segment proximal to a depolymerizing end, the density of the CPC with active Aurora B would be identical to that on an internal region (Fig. 3B). However, when Aurora B binds at a polymerizing end, by the time when it becomes activated, it would already have migrated to an internal position by treadmilling (Fig. 3C). If Aurora B-dependent INCENP phosphorylation also suppresses microtubule binding as shown in budding yeast [39,52], it would further restrict the diffusion-based accessibility of Aurora B that have been activated at distal locations. By this mechanism, activated Aurora B on microtubules will always be cleared from polymerizing ends.

On actively moving sister kinetochores that have established microtubule plus-ends, tension is observed at the trailing kinetochore attached to polymerizing microtubule ends, while tension is reduced on a leading kinetochore coupled to depolymerizing microtubules [53,54]. This may promote phosphorylation at the kinetochore attached to depolymerizing ends even after amphitelic attachment is achieved. However, on chromosomes aligned at the metaphase plate and undergoing oscillations, even kinetochores coupled to depolymerizing microtubules exhibit stronger Hec1-microtubule binding than those kinetochores whose tension is reduced by taxol or in monopolar spindles [17**]. As tension can selectively stabilize attachment to depolymerizing ends by an ATP-independent mechanism [7], the tension generated by bipolar attachment would be able to strengthen attachment to depolymerizing ends. Tension may also enhance the microtubule-mediated substrate masking effect, perhaps by increasing the microtubule-binding strength of the Aurora B targets. Once the balance shifts to dephosphorylation and stabilizes kinetochore attachment by recruiting the Ska complex and PP1, phosphorylation may not be able to re-occur [13].

The model may seem contradictory to a recent observation that suggests an importance of Aurora B at the centromere; inhibition of Haspin-dependent CPC recruitment at the centromere eliminates tension-dependent stabilization of Ndc80-microtubule binding, while it did not affect average binding affinity [17**]. However, as microtubule-dependent Aurora B activation facilitates centromeric localization of Aurora B [42–44], increasing the CPC pool at centromeres through microtubule-induced Aurora B activation may in turn facilitate phosphorylation of the kinetochore substrates, perhaps combined with a substrate-unmasking effect of basal phosphorylation via microtubule-bound Aurora B. Future studies including comparative measurement of Aurora B activation dynamics at microtubules and of the treadmilling rate, and biochemical analysis of substrate masking, should be able to validate these models.

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56. Kuhn J, Dumont S: Spindle assembly checkpoint satisfaction occurs via end-on but not lateral attachments under tension. *J Cell Biol* 2017, 216:1533–1542. [PubMed: 28536121] * It has not been clear if kinetochore attachment to the lateral side of microtubules can silence the spindle checkpoint. Through monitoring kinetochore localization of the checkpoint proteing Mad1, a signature of checkpoint activation, together with microtubules by live microscopy in rat-kangaroo PtK2 cells, this study demonstrates that lateral attachment can exert tension at the kinetochore, but this cannot trigger Mad1 dissociation. End-on microtubule attachment triggers Mad1 dissoation.

Figure 1. Aurora B-dependent error corrections of aberrant attachments.

A. Normal (amphitelic) and aberrant (syntelic and merotelic) attachments. **B.** Aurora B-mediated microtubule dissociation from aberrant attachments. Generation of an unattached kinetochore (yellow) activates the spindle checkpoint. **C.** Kinetochore attached to lateral side of microtubules can slide by the CENP-E motor toward the spindle equator [55]. Lateral attachment does not silence the checkpoint (yellow) [56*]. **D.** Conversion of lateral attachment to end-on attachment, and stabilization of amphitelic attachments, which silence the spindle checkpoint. Color brightness of each kinetochore indicates the level of Aurora B-dependent phosphorylation (or checkpoint strength).

Figure 2. Basic mechanistic principles behind selective stabilization of amphitelic attachments.
A. Geometric constraint. A pair of kinetochores linked through a geometric constraint (right) that interferes with attachment to microtubules coming from distal poles. The black box indicates the constraint, which may be promoted by the CPC at the centromere. In the absence of a geometric constraint (right), such as on paired homologous chromosomes during meiosis I, attachment from distal poles is enabled, resulting in merotelic attachment (and also syntelic attachment). **B.** Spontaneous microtubule turnovers. Each microtubule attachment has limited lifetime, and is spontaneously replaced by new attachment. A few aberrant attachments can be spontaneously replaced by correct attachment as the geometric constraint interferes with aberrant attachment. The turnover can be enhanced by Aurora B. **C.** Tension-induced enhancement of kinetochore-microtubule attachment (catch-bond mechanism). At a kinetochore under reduced tension (left), intrinsic microtubule binding is weak. At a kinetochore under tension (right), intrinsic microtubule binding is enhanced. Aurora B can weaken the microtubule-binding strength.

Figure 3. Members of the CPC; Aurora B, INCENP, Survivin and Borealin.

Aurora B interacts with INCENP at the IN-box domain, which allosterically activates Aurora B. Aurora B-dependent phosphorylation (yellow circles) at the Aurora B activation loop and on INCENP promotes full activation. The N-terminal CEN domain of INCENP interacts with Survivin and Borealin. This trimeric module targets the CPC to centromere-enriched histone phosphorylation marks; H3 phosphorylated at Thr3 recognized by the BIR domain of Survivin, and H2A phosphorylated at Thr120, which recruits Borealin through Sgo1 in a manner dependent on Cdk1-mediated phosphorylation at Borealin (orange circles). The SAH domain interacts with microtubules, while the PRD domain also supports microtubule binding, which is suppressed by Cdk1-dependent phosphorylation (orange circles). INCENP also interacts with HP1, which appears to facilitate substrate recognition.

Figure 4. Models: substrate masking by microtubules and treadmill-removal of Aurora B

A. Unattached kinetochore. Centromeric CPC enrichment activates Aurora B through autophosphorylation. Diffusion allows activated Aurora B (red) to access kinetochore substrates (orange dotted arrow), while its reaction is counteracted by PP2A at the kinetochore. The positively charged N-terminal tail of the Ndc80/Hec1 subunit of the Ndc80 complex (green) is recognized by Aurora B and microtubules. In the absence microtubules, Aurora B activated at the centromere can phosphorylate Ndc80.

B. Kinetochore attached to shrinking microtubule ends. The kinetochore is moving to a pole, and is under reduced tension. In the attached kinetochore, microtubules and Aurora B may compete to interact with the Ndc80 N-terminus, necessitating the microtubule-binding capacity of the CPC for effective Ndc80 phosphorylation (*substrate masking by microtubules*). The majority of the CPC with high Cdk1-dependent phosphorylation on INCENP (shown as orange circles) cannot bind to microtubules, but a small fraction of the CPC with reduced phosphorylation can bind to microtubules for a limited timeframe, during which Aurora B is activated. At the same duration, CPC with activated Aurora B comes closer to kinetochore substrates by treadmilling due to plus-end depolymerization, or stays close to the kinetochore (in the case of taxol-stabilized microtubules), facilitating accessibility of active Aurora B to its kinetochore substrates.

C. Kinetochore attached to growing microtubule ends. The kinetochore is moving away from a pole, and is under tension. The CPC with inactive Aurora B can be activated upon

microtubule binding, but by the time when Aurora B is activated, it will already have been treadmilled away from the kinetochore, reducing its accessibility to kinetochore substrates (*treadmill-removal*). The CPC activated by microtubules or the centromeric chromatin may diffuse into the cytoplasm, but the high Cdk1 activity limits the rebinding to microtubules within the kinetochore.

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