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Correcting aberrant kinetochore microtubule attachments: an Aurora B-centric view

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Abstract

The directed movement of chromosomes during mitosis and meiosis relies on microtubule-mediated connection between the spindle poles and kinetochores assembled on chromosomes. The molecular basis for the dynamic interaction between microtubules and kinetochores is just beginning to be unveiled. Here, focusing on the mitotic centromere kinase Aurora B, we review our current understanding of the signaling pathways that correct erroneous microtubule attachment at kinetochores. We evaluate several potential models that may explain how maloriented attachments can be recognized and processed by the Aurora B pathway.

Introduction

To direct chromosome movement during mitosis, the kinetochore, a proteinaceous structure assembled on centromeric DNA, dynamically captures microtubules. Importantly, all the microtubules attaching to a kinetochore on one chromatid must link to only one of the two spindle poles, while those attaching to its sister kinetochore must link to the opposite pole. Although the back-to-back orientation of the kinetochore arrangement may contribute to this correct, amphitelic attachment [1*], cells are equipped with machinery to correct the aberrant microtubule attachment geometries found in monotely, syntely and merotely (Figure 1) [2]. Furthermore, some of these aberrant attachments and lack of attachments activate the spindle assembly checkpoint (SAC, or mitotic checkpoint), which delays sister chromatid separation and mitotic exit [3].

How is an improper microtubule-kinetochore attachment recognized and repaired? Elegant work in budding yeast and grasshopper spermatocytes has demonstrated that tension at kinetochores/centromeres is a key factor in to stabilizing bi-oriented attachments [4,5]. Upon biorientation during mitosis, tension is generated by microtubules pulling at the kinetochore of each sister chromatid, held together by cohesion near or at centromeres. Indeed, studies in budding and fission yeast have shown that centromeric cohesion is important for accurate mitotic chromosome segregation [6,7]. In the case of a maloriented chromosome where tension is lost, a signaling cascade removes improper attachments, which in turn is believed to activate the SAC [3]. Multiple lines of evidence suggest that centromeric Aurora B kinase activity plays a major role in correcting these improper attachments and in SAC signaling [8,9]. Here, we will restrict our focus to the regulation of Aurora B activation with regard to the correction of

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improper microtubule attachment, and review several speculative models that may explain how this process is controlled.

Aurora B is required for correcting erroneous microtubule attachments at kinetochores

The chromosomal passenger complex (CPC), which contains the kinase Aurora B, INCENP, Dasra (also known as Borealin and CSC-1) and Survivin plays multiple roles at multiple places during mitosis (Figure 2) [10,11**]. Upon entry into mitosis, the CPC is first localized to both chromosome arms and the inner centromere, a region located between sister kinetochores. As the cell cycle progresses to metaphase, the amount of CPC localized to the chromosome arms decreases and it is mainly detected at the inner centromere [12]. When sister chromatids separate in anaphase, the CPC dissociates from centromeres and relocalizes to the spindle midzone.

During metaphase, the CPC is critical for the recruitment of a growing number of proteins to the kinetochore and centromere: outer kinetochore proteins, including those involved in SAC signaling (Mad1, Mad2, Bub1, BubR1, Mps1 and Cenp-E) [13–15]; proteins responsible for microtubule-kinetochore interactions (Cenp-E, Ndc80, Knl1, Mis12, Zwilch, $p150^{Glued}$, MCAK, Dam1 and Plk1) [16–21]; and other inner centromeric proteins such as the Shugoshin family proteins (Sgo1, Sgo2) and MCAK [20,22,23*]. Therefore, the CPC is one of the most upstream regulators of centromere/kinetochore function.

If CPC function is compromised, chromosomes with syntelic and merotelic attachments are frequently observed [14,24,25] due to the failure of improper connections to detach [8,9]. Aurora B-dependent phosphorylation of some key substrates is believed to facilitate the destabilization of aberrant attachments. Accordingly, both the CPC and activated Aurora B are preferentially enriched at merotelically attached kinetochores. This has been determined by using phospho-specific antibodies against the active forms of Aurora B and INCENP [26]. Several studies also indicate that Aurora B-mediated phosphorylation is less abundant on kinetochores at metaphase than at prometaphase when proper attachment has not yet occurred [17,18,27].

The mechanisms by which Aurora B kinase activity destabilizes improper microtubule attachments are not completely understood. However, several key substrates have been elucidated. The Ndc80/Hec1 complex is a major attachment module for microtubules at the outer kinetochore. In the absence of tension, it was proposed that Aurora B phosphorylates Ndc80, which decreases its affinity for microtubules (Figure 2) [28,29]. In addition, Dam1, a protein that allows kinetochores to track depolymerizing plus ends of microtubules in budding yeast, is negatively regulated by Ipl1 (Aurora B)-mediated phosphorylation [30].

Aurora B also regulates the microtubule-depolymerizing enzyme MCAK [17–19]. Interestingly, this appears to be both positive and negative regulation as Aurora B-mediated phosphorylation of MCAK suppresses its depolymerizing activity, but also controls its accumulation at centromeres [17–19]. Although MCAK accumulation can lead to microtubule destabilization, it remains unclear how Aurora B-mediated suppression of MCAK activity contributes to this process [31].

Regulators of the Aurora B pathway

The Aurora B pathway can be regulated by controlling the level of kinase activation directly or by controlling the balance of phosphorylation and dephosphorylation of its substrates. Biochemical and structural evidence suggest that for Aurora B to be fully active, it must

phosphorylate residues at the C-terminus of INCENP. However, it is predicted that the phosphorylation sites on INCENP are improperly oriented with respect to the active site of the kinase for phosphorylation to occur in *cis* and that this reaction is carried out in *trans* [32]. Indeed, high local concentrations of the CPC lead to full activation of Aurora B kinase, even in the presence of counteracting phosphatase activity [33*]. Furthermore, both chromatin and microtubules also lead to activation of the Aurora B kinase pathway, possibly through a similar mechanism of enrichment and trans-autoactivation and/or yet unknown allosteric mechanisms [33*,34]. Interestingly, another inner centromeric protein, TD-60, was shown to enhance the activation of Aurora B by microtubules [35*]. As detailed in the models below, these activities are key to our understanding of CPC function at the centromere during mitosis (Figure 2).

Two protein kinases recruited to kinetochores can also directly stimulate the kinase activity of Aurora B (Figure 2). Chk1, a protein kinase involved in the DNA damage checkpoint, is transiently localized to kinetochores and controls kinetochore microtubule attachments by activating Aurora B [36*]. Mps1, a protein kinase required for the SAC, controls kinetochore microtubule attachment and correction [37*,38**]. Mps1 stimulates Aurora B activity via phosphorylation of Borealin (Dasra B), modifications critical to error correction of microtubule attachments [38**]. These results suggest that Mps1 and Chk1 stimulate Aurora B activity to correct maloriented attachments in vertebrate cells.

In addition to these positive regulators of Aurora B, a number of negative regulators are believed to be important for limiting Aurora B activity. Several pieces of evidence suggest that protein phosphatase 1 (PP1) is the major counteracting phosphatase of the Aurora B pathway (Figure 2) [16,39,40]. In vertebrates, PP1α and PP1γ xare localized to the outer kinetochore [41,42], where they may remove Aurora B-dependent marks. PP1 probably serves two functions with respect to Aurora B: setting a threshold of kinase activity to counteract random fluctuations in Aurora B activity and allowing for rapid re-attachment of microtubules after removal of incorrectly attached ones.

Another potentially important negative regulator of the Aurora B pathway is the kinetochore kinase, BubR1 (Figure 2). In BubR1-depleted cells, the majority of kinetochores fail to attach to microtubules. However, this attachment defect is due in part to an increase in Aurora B activity upon BubR1-depletion [43,44]. Since Aurora B is required for the phosphorylation and kinetochore localization of BubR1 [14,25,45], BubR1 could act as a negative feedback regulator of the Aurora B pathway.

Potential mechanisms by which Aurora B regulates connections between kinetochores and microtubules

What is the mechanism by which tension and/or microtubule attachment status is sensed and how is this state translated into regulation of Aurora B kinase activity? Here we discuss three mechanisms by which the presence or absence of tension/attachment might modulate the Aurora B kinase pathway, with a focus on correction of aberrant microtubule attachments (Figure 3).

Model 1:Tension-regulated separation of Aurora B from its kinetochore substrates

It has been proposed that it is the physical distance between Aurora B and its kinetochore substrates that determines whether microtubule-kinetochore connections are maintained [8, 18]. When sister chromatids are under tension, the distance between pairs of kinetochores is increased relative to a relaxed state. Aurora B remains at the inner centromere, and therefore its kinetochore substrates are no longer co-localized with the kinase (Figure 3) [18]. Under this model, this leads to a situation in which phosphorylation of key kinetochore substrates (e.g.

Ndc80) is low and microtubule-kinetochore interactions are stabilized. In turn, PP1, localized to kinetochores in metaphase, dephosphorylates kinetochore substrates to maintain correctly attached microtubules [41,42]. Conversely, when there is little or no tension, Aurora B is physically closer to its substrates and phosphorylation is high, thus leading to destabilization. Reinforcement of this state might occur through the action of kinetochore kinases such as Mps1 and Chk1, which directly activate Aurora B [38**] [36*].

This model assumes that tension-dependent changes in distance between Aurora B and its substrates are enough to prevent interaction. In mammalian cells, differences of roughly 1–3 μm are seen between kinetochores under tension and those in a relaxed state [46]. As the CPC has been shown to be a highly elongated complex with maximum lengths of up to ~40–50 nm [47], it is possible that tension can physically separate Aurora B from its substrates. However, for this model to work, diffusion of the CPC must be very low. As there are conflicting reports on the dynamics of the CPC at the centromere [48–50], this point needs further investigation.

Furthermore, this model may not explain Aurora B-dependent error correction mechanisms during meiosis. In mouse spermatocytes, the CPC remains closely associated with kinetochores during metaphase I and metaphase II [51,52]. Upon progression from prometaphase II to metaphase II, the inner-centromeric fraction of the CPC diminishes while the adjacent kinetochore fraction remains [51]. In addition, this model does not readily explain why full enrichment of the CPC at inner centromeres may not be absolutely essential for the error correction process in vertebrate cells [15,53], while it was proposed that Sgo2-dependent centromeric localization of the CPC is critical for this process in fission yeast [23*]. To further validate this model, it is important to quantify the spatial distribution of Aurora B-dependent phosphorylation at centromeres and kinetochores in the absence and presence of tension. Recent developments such as the Aurora B activity FRET sensor and chromatin micro patterning will be helpful in addressing this issue [54**].

Model 2: Microtubule-dependent regulation of Aurora B

Microtubule-mediated regulation of Aurora B may play a major role in transducing the force of microtubule-kinetochore connections to Aurora B activity [55]. In budding yeast, there is recent evidence that the CPC may act as a molecular bridge between microtubules and the centromere. This suggests that the CPC is both the tension-sensor and the master-regulator of error correction [55], though it remains possible that the microtubule-binding domain may have other functions. In this model, under conditions of low tension, Aurora B activity is high leading to a weakening of microtubule attachments. Upon correct bi-orientation, tension is now applied to the CPC “bridge” which in turn causes a conformational change that leads to inhibition of the kinase.

This model is simple and direct in that there is a discrete conformational change that is transformed into a chemical signal that regulates microtubule attachment. However, it is not clear whether this mechanism is feasible in metazoans where the CPC is localized to the inner centromere and therefore may not directly interact with microtubules at the outer kinetochore. One possibility is that the forces of tension are transduced through multiple components that ultimately impinge upon the CPC and activate it in the manner detailed above. Alternatively, the simple act of binding of microtubules to the CPC could be responsible for Aurora B activation, assuming that microtubules interact with the CPC specifically at centromeres with maloriented kinetochores (Figure 3) [35]. Merotelic attachments, although still localized near the metaphase plate, have been suggested to cause an increased frequency of microtubule plus ends at the inner centromere [56].

Interestingly, removal of the domain of INCENP that is required for microtubule-mediated Aurora B activation (Boo Shan Tseng and H. F., unpublished results) does not affect its ability

to correct improper attachments in HeLa cells [57*]. Rather, this mutant is defective in activating the SAC in response to unattached kinetochores [57*]. To make sense of this conflicting data, it is important to show that the CPC-microtubule interaction occurs at maloriented attachments *in vivo* and that this interaction plays a role in error correction. In addition, biophysical studies are needed to demonstrate whether Aurora B kinase activity can be regulated by microtubule-dependent tension.

Model 3: Modulation of Aurora B activity through structural changes in centromeric chromatin

Here we consider a third model, in which changes in the centromeric chromatin structure regulate Aurora B activity, as previously indicated [8]. Tension generated by microtubules pulling on kinetochores can provide enough force to potentially unwind nucleosomes at the centromere (discussed in [58]). Therefore, tension might affect the distribution of CPC molecules at the centromere. We propose that under low tension, chromatin is in a compact state resulting in a high effective concentration of the CPC. This may increase the likelihood that one CPC molecule phosphorylates another, which has been shown to lead to sustained activation of the kinase. When the centromere is under tension, this mechanism may be suppressed due to a decreased local concentration of the CPC and/or physical disruption of its oligomerization state. Monitoring the dynamics of CPC inter-molecular interactions under conditions of both high and low tension will aid in the validation of this model.

Alternatively, chromatin-mediated activation of Aurora B may be sensitive to the structure or the topological orientation of the DNA. The fact that topoisomerase II is required for Aurora B-mediated phosphorylation indicates that unresolved topological constraints could interfere with Aurora B activation [59**]. Strikingly, the inner centromeric ATPase PICH controls topoisomerase II-dependent decatenation of centromeric DNA during mitosis, and is required for proper chromosome alignment [60*,61**] (Lily Wang and Erich Nigg, personal communication). Thus, the topological status of centromeric DNA may be carefully regulated during mitotic progression. A reconstituted CPC-chromatin system is needed to elucidate whether changes in chromatin structure/topology can alter Aurora B kinase activity.

Conclusions

It is readily apparent that Aurora B represents a hub, in which many pathways converge to transduce the mechanical forces imposed by microtubule-kinetochore attachments into chemical signals that regulate such attachments. We have yet to understand the molecular nature by which improper attachments are removed and reestablished, but we have some valuable clues. It is important to remember that the Aurora B kinase pathway interacts with several centromeric/kinetochore kinases (Bub1, BubR1, Mps1 and Plk1) as well as phosphatases (PP2A-Sgo1, PP2A-Sgo2 and PP1) (Figure 2). The extent to which each of these effectors regulates Aurora B kinase activity and localization also depends on the nature of the microtubule-kinetochore connection. Therefore, a small perturbance to this system could result in an amplified Aurora B response due a complex network of feedback loops. A full understanding of the kinetic parameters governing each interaction in this complex network, in conjunction with computational modeling, will be key to tackling this exciting problem.

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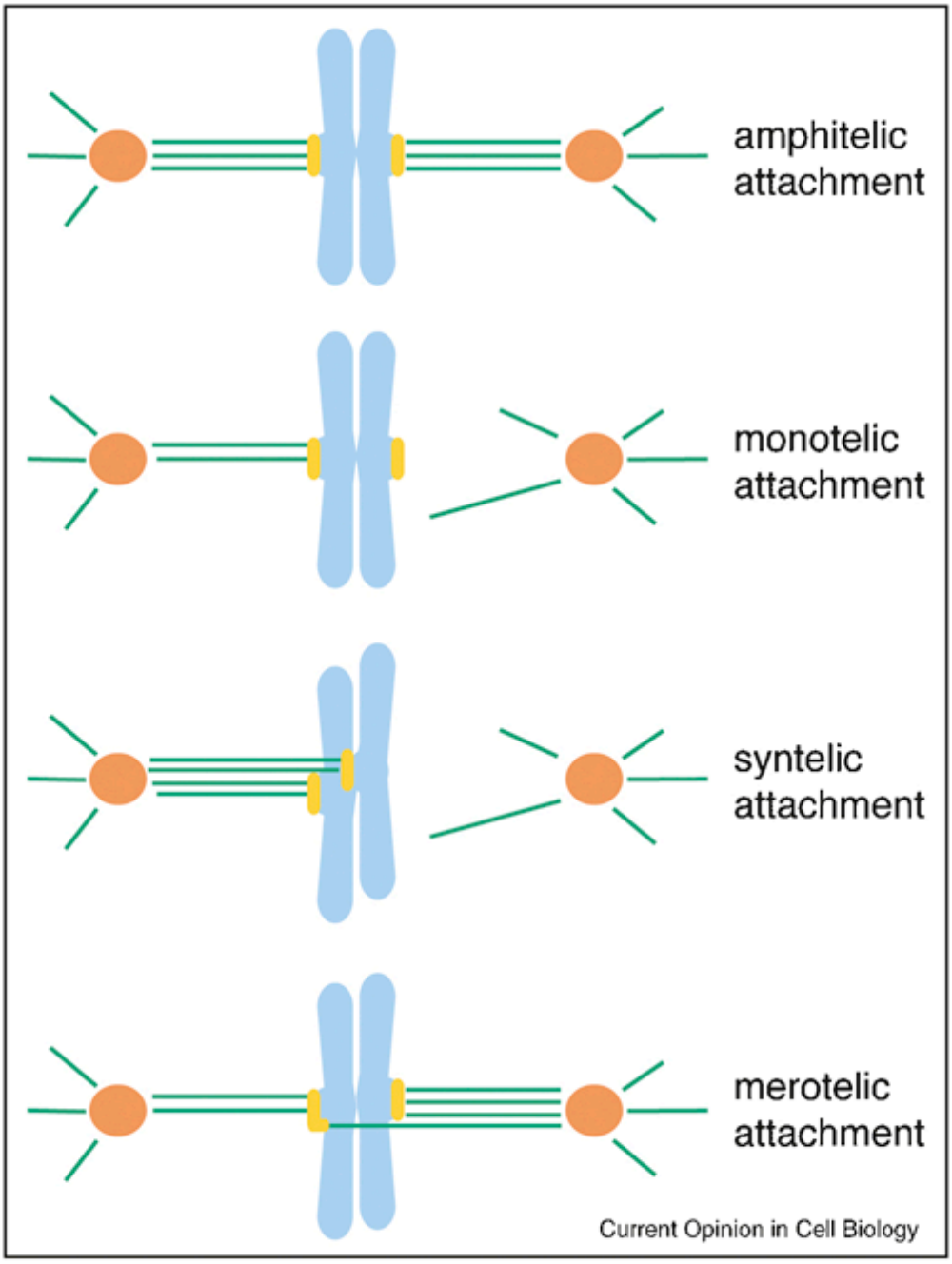

Figure 1.
Classification of kinetochore microtubule attachments.
Amphitelic attachment: this is the correct attachment, in which all the microtubules attached to a kinetochore connect one spindle pole, while all those attached to its sister kinetochore link to its opposite pole. Monotelic attachment: a kinetochore attaches to microtubules that link to one spindle pole, while its sister kinetochore does not attach to any microtubules. Syntelic attachment: both sister kinetochores are linked to the same pole by microtubules. Merotelic attachment: a kinetochore attaches to microtubules from more than one spindle pole, a situation that results in a lagging chromosome during anaphase. Kinetochores are in yellow, microtubules in green, chromosomes in light blue and centrosomes in orange.

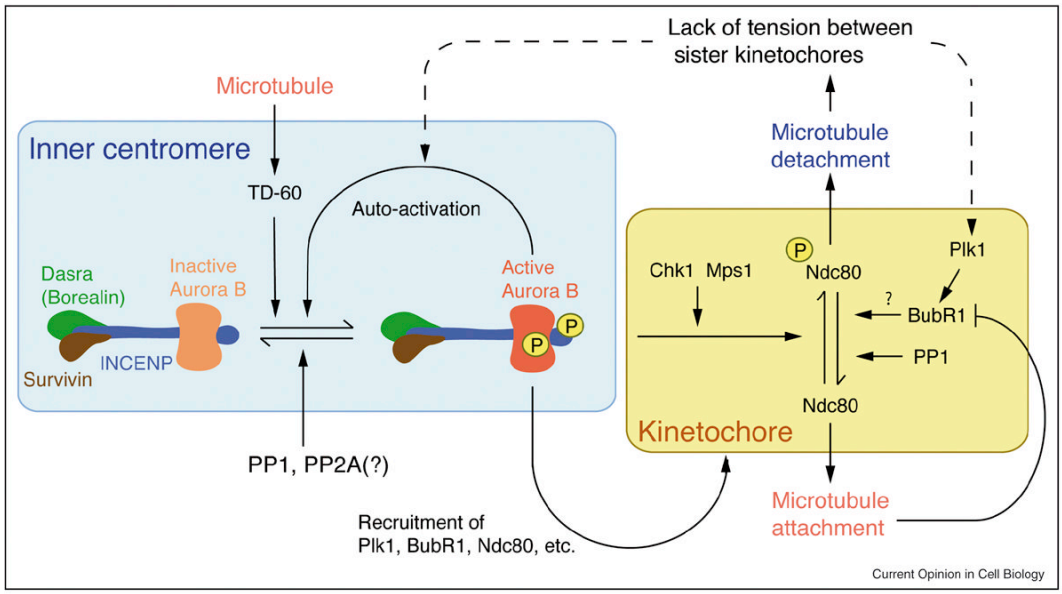

Figure 2.

Aurora B pathway control of microtubule attachment.

The CPC, composed of Aurora B, INCENP, Dasra (Borealin) and Survivin, is localized to inner centromre, where it signals to correct mal-oriented kinetochore-microtubule attachments. Aurora B can be autoactivated by phosphorylation *in trans*, but the reaction is inhibited by phosphatases. Upon loss of tension between sister chromatids, inner centromeric Aurora B is activated, where activation may occur directly or indirectly. At the kinetochore, Aurora B phosphorylates Ndc80, leading to destabilization of microtubules at kinetochores. Lack of tension activates the Plk1 dependent phosphorylation of BubR1, a modification which is critical for microtubule attachment [62]. Once microtubule attachment is established, BubR1 is inactivated [63]. Upon bipolar attachment, the Aurora B pathway and the Polo pathway are inactivated, possibly through the action of PP1.

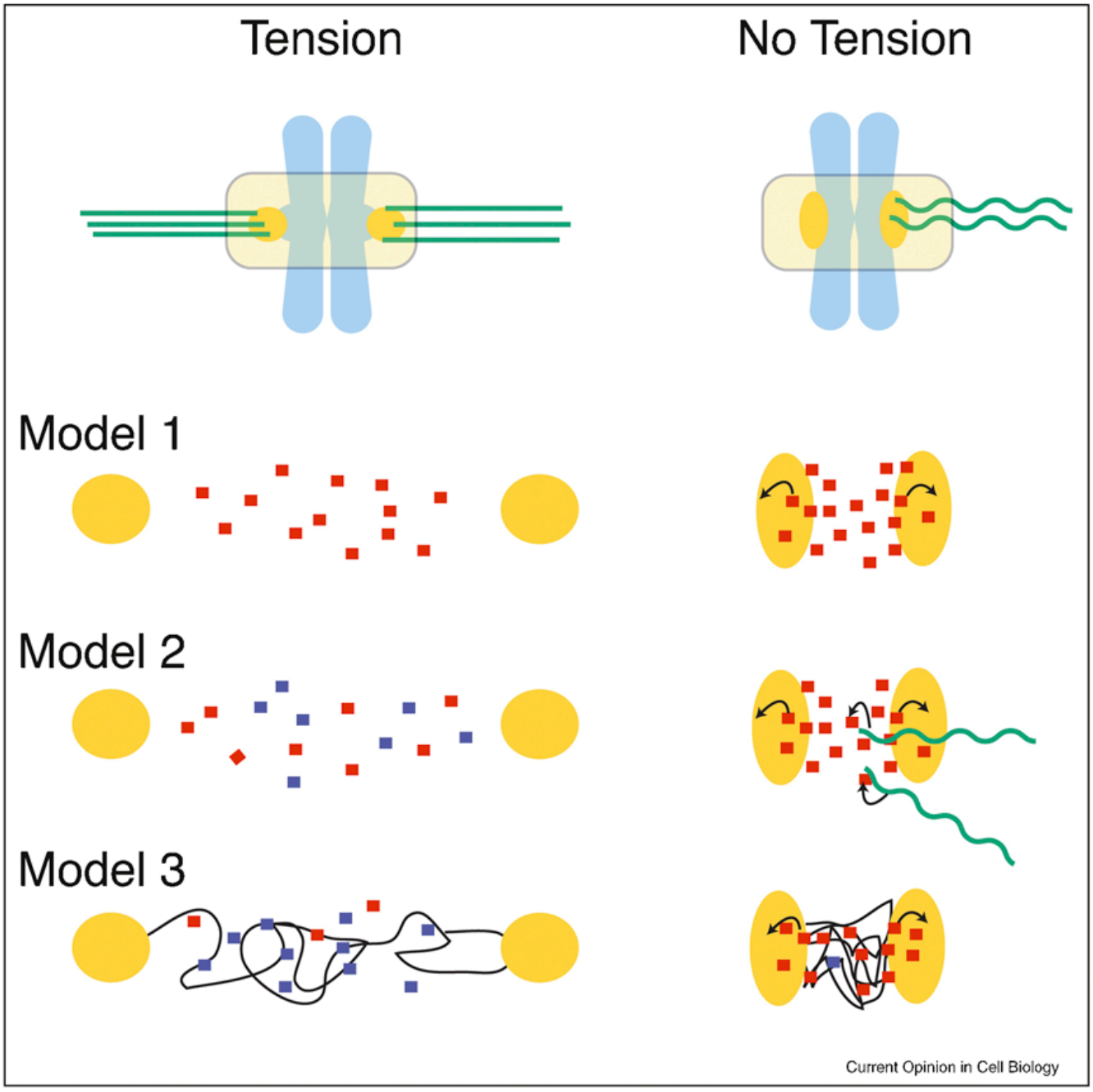

Figure 3.

Three models of how the Aurora B pathway is controlled by microtubule attachment or tension. Each model is a simplified enlargement of the inter-kinetochore regions of chromosomes under tension or in a relaxed state. Kinetochores are in orange, microtubules in green, and chromosomes are in light blue. Arrows indicate activation.

Model 1: ***Tension-regulated separation of Aurora B from its kinetochore substrates***

The distance between active Aurora B at inner centromeres and its kinetochore substrates increases upon bioriented attachment. This increased distance would decrease the likelihood of Aurora B binding kinetochore substrates, which is reinforced by PP1 phosphatase.

Model 2: ***Microtubule-dependent regulation of Aurora B***

Inner centromeric Aurora B on misaligned chromosomes is more accessible to microtubules than at the metaphase plate. Interactions with microtubules stimulate the kinase activity of Aurora B resulting destabilized microtubule attachments.

Model 3: ***Modulation of Aurora B activity through structural changes in centromeric chromatin***

Assuming that the CPC is activated by chromatin through a clustering mechanism, the lack of tension may cause a compaction of chromatin at the centromere leading to kinase activation. However, under tension, the chromatin fibers are stretched resulting in a lower effective concentration of the CPC and thus activity. Chromatin fibers are modeled as thin black lines.