Regulation of kinetochore–microtubule attachments by Aurora B kinase

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Abstract

Accurate segregation of chromosomes in mitosis requires that spindle microtubules attach sister kinetochores to opposite poles of the mitotic spindle (biorientation). To achieve biorientation of all chromosomes, incorrect attachments are selectively destabilized, providing a fresh opportunity to biorient, whereas correct attachments are stabilized. Tension across the centromere may be the signal that distinguishes different attachment states, as spindle microtubules pull bioriented sister kinetochores in the opposite direction. Destabilization of incorrect attachments requires the Ipl1/Aurora B kinase, which phosphorylates kinetochore substrates that directly interact with microtubules. The present review focuses on how Aurora B regulates attachments in response to centromere tension.

Introduction

The accurate segregation of chromosomes during cell division is essential to maintain genome stability. In mitotic divisions an exact copy of the genome is propagated from the mother cell to the two daughters, and in meiosis the number of chromosomes is halved to produce gametes that contain exactly one chromosome from each pair of homologous chromosomes in the parent. The present review will focus on mitotic divisions, but meiotic divisions probably use similar mechanisms. In eukaryotic cells, a microtubule-based structure, the mitotic spindle, generates forces that pull the sister chromatids in opposite directions before the cell cleaves in between the separated chromosomes. A kinetochore assembles at the centromere of each chromosome to mediate interactions with spindle microtubules. To ensure accurate segregation, every pair of sister kinetochores must attach to microtubules from opposite poles of the spindle, referred to as biorientation. How this configuration is achieved for every pair of replicated chromosomes is a long-standing question in cell biology.

Kinetochores may initially bind microtubules in any configuration. Although there is a bias towards biorientation due to geometric constraints [1,2], it is unlikely that all chromosomes will do so initially. Kinetochore–microtubule attachments must therefore be regulated. If incorrect attachments are destabilized, while correct attachments are stabilized, all kinetochores should eventually reach the correct attachment state in a trial-and-error process, as destabilization provides a fresh opportunity to biorient (reviewed in [3]). In principle, a mechanism to selectively stabilize only correct attachments would be sufficient to eventually guarantee biorientation of all chromosomes. An appealing model is that the stability of attachments might depend on tension across the centromere. Bioriented attachments are under tension because spindle microtubules pull sister kinetochores in opposite directions. The first direct experimental evidence for the tension model came from classic micromanipulation experiments in insect spermatocytes in Meiosis I [4]. Unipolar attachments, in which both half-bivalents are attached to the same spindle pole, are unstable and reorient within a few minutes. If tension is applied with a micromanipulation needle, however, the unipolar attachments are stable and fail to reorient until tension is released. These experiments demonstrated that tension selectively stabilizes kinetochore–microtubule attachments, thereby ensuring that all chromosomes eventually biorient.

Regulation of attachments by Aurora B kinase

Although the micromanipulation experiments laid the foundation for a model explaining how biorientation can be achieved, the molecular details of how attachments are regulated were unclear. An important component of this regulation was first identified based on an ipl (increase-in-ploidy) phenotype in budding yeast [5]. The Ipl1 kinase was subsequently shown to be required for accurate chromosome segregation, to phosphorylate kinetochore substrates and to regulate microtubule binding to kinetochores [6–8]. Further work in budding yeast showed that Ip1 is required to activate the spindle checkpoint in response to loss of tension but not loss of microtubule attachment, suggesting that Ipl1 might be involved in sensing tension across the centromere [9]. An elegant series of experiments showed that if chromosome replication is prevented, which precludes the formation of bioriented attachments, the connections between kinetochores and spindle pole bodies are unstable in the presence of wild-type Ipl1. If Ipl1 is mutated, however, these connections are stabilized, which suggests that one function of Ipl1 is to promote turnover of attachments in the absence of tension [10].

Key words: Aurora B kinase, biorientation, chromosome, kinetochore–microtubule attachment, mitosis, phosphatase.

Abbreviations used: CENP-B, centromere protein B; FRET, fluorescence resonance energy transfer; INCENP, inner centromere protein; MCAK, mitotic centromere-associated kinesin; PP1, protein phosphatase 1.

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In mammalian cells, the Ipl1 homologue, Aurora B, is also required for accurate chromosome segregation. If Aurora B is inhibited using small-molecule inhibitors, incorrect attachments are stabilized, for example with both sister kinetochores attached to a single spindle pole [11]. Activation of Aurora B by removing the inhibitor leads to correction of these attachment errors by selectively destabilizing the incorrect attachments, while correct attachments remain stable [12]. Together, these studies suggest a model in which Ipl1/Aurora B phosphorylates kinetochore substrates in the absence of tension to destabilize incorrect attachments and allow reorientation. Aurora B localizes to the inner centromere, close to the kinetochores, which places it close to the site where regulation is required.

Substantial progress has been made towards understanding the molecular details of how Ipl1 phosphorylation regulates interactions between kinetochores and microtubules (reviewed in [13]). Multiple Ipl1 substrates have been identified at the kinetochore, including the Dam1 and Ndc80 complexes in budding yeast [7]. These two complexes are of particular interest because they can couple microtubule polymerization and depolymerization to force production and movement of a bead *in vitro*, suggesting that they do the same for chromosomes *in vivo* [14–17]. The Dam1 complex can oligomerize *in vitro* to form a ring that encircles a microtubule [18,19], which would provide an appealing mechanism for tracking a depolymerizing microtubule end, although ring formation may not be required for its function [20,21]. As suggested by structural studies, Ipl1 phosphorylation reduces the affinity of purified Dam1 complexes for microtubules and increases the frequency of detachment of Dam1 complexes diffusing along microtubules [20,22]. Dam1 is essential in budding yeast, but no clear homologues have been identified in vertebrates. The Ndc80 complex is conserved from yeast to mammals and is essential for microtubule binding to the kinetochore (reviewed in [23]). The complex includes a long coiled-coil region with globular domains at both ends, one oriented towards the inner kinetochore and the other associated with microtubules [24,25]. Phosphorylation of the Ndc80 complex by Ipl1/Aurora B reduces the affinity for microtubules [26,27]. Expression of a mutant Hec1, one member of the mammalian Ndc80 complex, in which Aurora B phosphorylation sites have been mutated, leads to stabilization of incorrect attachments and chromosome segregation errors [28]. Multiple lines of evidence therefore support a model in which phosphorylation of kinetochore substrates by Ipl1/Aurora B destabilizes kinetochore microtubules, and this regulatory mechanism appears to be conserved from yeast to mammals.

Another class of Aurora B substrates that may be important for regulating kinetochore–microtubule attachments in vertebrates is the kinesin-13 family, which catalyse depolymerization at the ends of microtubules [29]. MCAK (mitotic centromere-associated kinesin; Kif2c) is the best-characterized member of this family. Aurora B phosphorylates multiple sites on MCAK to regulate both its activity and its localization to the inner centromere or kinetochore [30–33]. Both MCAK and another kinesin-13 family member, Kif2b, regulate kinetochore–microtubule dynamics and contribute to correcting chromosome attachment errors [34,35]. Whereas direct regulation of Kif2b by Aurora B has not been demonstrated, Aurora B kinase activity is required for Kif2b localization to kinetochores [35]. Thus regulation of the activity and localization of kinesin-13 proteins is another mechanism by which Aurora B activity might destabilize incorrect attachments.

Aurora B as tension sensor

The details of how phosphorylation of Aurora B substrates destabilizes kinetochore–microtubule attachments are becoming clearer, but the question how the regulation is selective, so that only incorrect attachments are destabilized remains unanswered. The tension model predicts that phosphorylation is sensitive to mechanical forces at the centromere, with high phosphorylation when tension is low, which would destabilize incorrect attachments, and low phosphorylation when tension is high. Two general models were proposed that might explain tension sensitivity [10]. In the first model, tension could induce a conformation change either in the kinase itself or in a regulatory protein, for example through interactions with centromeric heterochromatin, which would directly inhibit the kinase activity (Figure 1A). As a precedent for this kind of regulation, mechanosensitive ion channels are gated by changes in membrane tension [36]. In the second model, kinase activity is constant, but tension changes the distance between kinase and substrate by pulling the kinetochores away from the kinase at the inner centromere (Figure 1B). Increased spatial separation of kinase from substrate would decrease phosphorylation of kinetochore substrates when tension is high without directly inhibiting the kinase. Both models predict tension-sensitive phosphorylation of kinetochore substrates, but they make different predictions about phosphorylation of substrates closer to the inner centromere. Phosphorylation of a centromere substrate is tension sensitive in the first model but independent of tension in the second model.

Based on the predictions of the two models for tension-sensitivity, measuring the phosphorylation state of Aurora B substrates at different sites would help distinguish between the two. One approach would be to use phospho-specific antibodies to examine phosphorylation of one Aurora B substrate at the kinetochore and another substrate at the centromere. Interpretation of these results might be complicated, however, by comparing two different substrates. Also, it is difficult to analyse phosphorylation dynamics by this method, since the analysis is in fixed cells. Another approach is to measure phosphorylation changes using a biosensor, which reports on phosphorylation of a constant substrate and can be targeted to different sites. Several strategies for designing phosphorylation sensors based on FRET (fluorescence resonance energy transfer) have been described, which allow changes in phosphorylation to be tracked by fluorescence microscopy in living cells with high temporal and spatial resolution (reviewed in [37]). One of these designs was adapted

Figure 1 | Models for tension-sensitive regulation of kinetochore microtubule attachments by Aurora B

In the first model (**A**), Aurora B kinase activity is tension sensitive, so the kinase is inhibited at bioriented centromeres. In the second model (**B**), the kinase is constitutively active, but tension leads to spatial separation of the kinase at the inner centromere from kinetochore substrates, which are then dephosphorylated. 'P' indicates phosphorylation of kinetochore substrates.

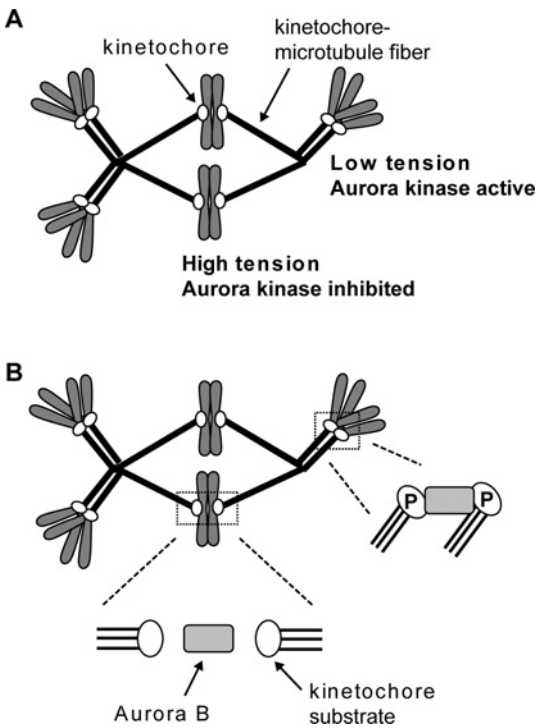
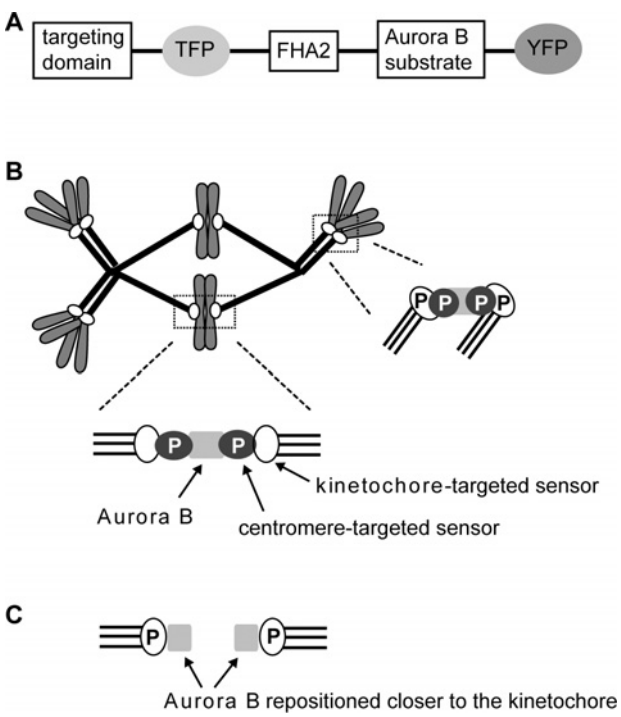


Figure 2 | Phosphorylation of an Aurora B substrate at different sites

(**A**) Schematic diagram of the FRET-based phosphorylation sensor, which includes teal fluorescent protein (TFP) as a FRET donor, the FHA2 phosphopeptide-binding domain, an Aurora B substrate peptide and yellow fluorescent protein (YFP) as a FRET acceptor. Phosphorylation of the substrate peptide induces a conformation change through binding to the FHA2 domain, which changes the efficiency of energy transfer between TFP and YFP (see [50] for the general design and [39] for further details of the Aurora B sensor). (**B**) An Aurora B phosphorylation sensor targeted to the kinetochore is phosphorylated (indicated by 'P') when tension is low and dephosphorylated when tension is high. The same sensor targeted to the centromere is constitutively phosphorylated. (**C**) If Aurora B is repositioned closer to the kinetochore, then the kinetochore-targeted sensor is also constitutively phosphorylated. The schematic diagrams in (**B**, **C**) summarize results from [39].



to create a sensor that reports on changes in phosphorylation of an Aurora B substrate [38] (Figure 2A). The sensor was targeted to the centromere using the DNA-binding domain of the centromere protein CENP-B (centromere Protein B) or to the kinetochore using full-length Mis12, a core component of the kinetochore. These targeted sensors were used to test the predictions of the two models, using fluorescence microscopy to examine changes in phosphorylation of an Aurora B substrate at different sites [39]. The kinetochore-targeted sensor is phosphorylated when tension is low and dephosphorylated when tension is high, as predicted by the tension hypothesis. Furthermore, the centromere-targeted sensor is constitutively phosphorylated, independent of tension (Figure 2B). Measurements of phosphorylation dynamics during the correction of attachment errors show that phosphorylation of kinetochore substrates occurs on the same time scale as the error correction process. These findings are consistent with the second model, in which the kinase is always active and phosphorylation of kinetochore substrates depends on the spatial separation of kinase from substrate.

According to the spatial separation model, the localization of Aurora B to the inner centromere establishes the distance to kinetochore substrates. The model predicts that repositioning Aurora B from the inner centromere closer to the kinetochore will lead to both increased phosphorylation of kinetochore substrates and destabilization of microtubule attachments. A strategy to manipulate the position of the kinase was designed based on its association with the chromosome passenger complex, which includes INCENP (inner centromere protein), survivin and borealin. This complex is required both for Aurora B targeting to the inner centromere and for normal kinase activity (reviewed in [40]). By removing an N-terminal domain of INCENP, which is required for centromere targeting, and replacing it with other targeting sequences, INCENP fusion proteins were created that would bind endogenous Aurora B and target it to other sites at the centromere or kinetochore [39]. Repositioning Aurora B closer to the kinetochore, using an INCENP fusion protein with the centromere-binding domain of CENP-B,

increases phosphorylation of the FRET-based sensor at the kinetochore and destabilizes kinetochore microtubules (Figure 2C). If Mis12 is used in the INCENP fusion protein, Aurora B targets right to the kinetochore and destabilizes microtubules very effectively. These results indicate that the tension-sensing mechanism fails when Aurora B is positioned closer to kinetochore substrates, leading to constitutive phosphorylation and destabilization of microtubule attachments.

The spatial separation model explains how mechanical forces at the centromere may regulate kinetochore–microtubule stability. Forces exerted by spindle microtubules pull bioriented sister kinetochores in opposite directions, which increases the distance between the inner centromere, where Aurora B localizes, and the outer kinetochore, where microtubules bind. In this configuration the kinase does not efficiently phosphorylate substrates at the outer kinetochore, such as the Ndc80 complex, so attachments are stabilized. In the absence of such forces, kinetochore substrates are phosphorylated because they are in close proximity to Aurora B at the inner centromere, leading to destabilization of attachments. Other mechanisms may also contribute to regulation of Aurora B activity, including kinase enrichment at incorrect attachments, direct interactions with microtubules and chromatin structural changes that might modulate kinase autoactivation [41–44]. Destabilization provides a fresh opportunity to biorient, which may be facilitated by a mechanism that transports mono-oriented chromosomes to the spindle equator, which increases the likelihood of a free kinetochore capturing a microtubule from the opposite pole [45]. Selective destabilization in the absence of tension is therefore an integral component of a trial-and-error mechanism that ultimately promotes biorientation of all chromosomes.

What about phosphatases?

Most studies addressing regulation of kinetochore–microtubule attachments by phosphorylation have focused on kinases, particularly Aurora B. Phosphorylation dynamics depend, however, on the balance of kinase and phosphatase activities. For example, if phosphorylation of kinetochore substrates such as the Ndc80 complex destabilizes incorrect attachments, then the same substrates should get dephosphorylated after biorientation is achieved, so that correct attachments are stable. Genetic evidence in budding yeast suggests that PP1 (protein phosphatase 1) opposes Aurora B kinase activity [8,46,47]. In HeLa cells the PP1γ isoform localizes to the kinetochore [48] and thus might be poised to dephosphorylate kinetochore substrates. How PP1γ is targeted to the kinetochore is not known, and the importance of this localization for regulating kinetochore–microtubule attachments has not been tested. A simple model is that Aurora B kinase activity at the kinetochore is regulated by tension, as discussed above, whereas phosphatase activity at the kinetochore is constant. Alternatively, modulation of the phosphatase could provide an additional layer of regulation. Whether PP1 activity or kinetochore binding is regulated is an open question. PP2A (protein phosphatase 2A) also localizes to the centromere (reviewed in [49]), but it is not known whether it has a role in regulating kinetochore microtubules. A more comprehensive understanding of mechanisms that control chromosome segregation will have to incorporate both kinases and phosphatases.

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