Correction mechanisms of erroneous kinetochore-microtubule attachments in chromosome segregation

Banafsheh Etemad

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By: Banafsheh Etemad
Studentnumber: 3018997
Email: b.etemad@students.uu.nl

Supervised by: Dr. G. Kops
Faculty of medicine, University Medical Center Utrecht
Department of Physiological Chemistry
Email: g.j.p.l.kops@umcutrecht.nl

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Introduction

During the process of chromosome segregation, duplicated DNA of one cell has to be equally segregated between daughter cells to allow their proper development. Chromosome segregation is mediated by the mitotic spindle, a structure consisting of microtubules that emanate from two opposing microtubule organizing centers called the centrosomes (Figure 1A). The spindle microtubules drive chromosome segregation by connecting to the sister chromatids and pulling them to opposing sides of the dividing cell. Chromatids associate with microtubules via the kinetochore, a network of proteins assembled on a specialized stretch of chromatin known as the centromere (Welburn and Cheeseman, 2008). Each sister chromatid contains one kinetochore that can bind up to ~30 microtubules from multiple directions and configurations (Dong *et al.*, 2007; McEwen *et al.*, 1997). Upon segregation, the cell will divide in a plane perpendicular to the direction of segregation, creating two cells with equal amounts of DNA. Proper chromosome segregation is only achieved when each chromatid is exclusively connected to microtubules from only one spindle pole and when sister chromatids are bound to opposite poles. This is called bi-orientation or amphitely of chromosomes and is a tightly regulated process (Figure 1B). Segregation of sister chromatids to one side of the cell, instead of to opposite sides, leads to aneuploidy, a state wherein cells contain an abnormal number of chromosomes. Mild levels of aneuploidy contribute to tumor formation and severe levels cause cell death. Aneuploidy has been suggested to be a consequence of merotelic orientation of kinetochores. In this orientation, a single kinetochore is connected to both spindle

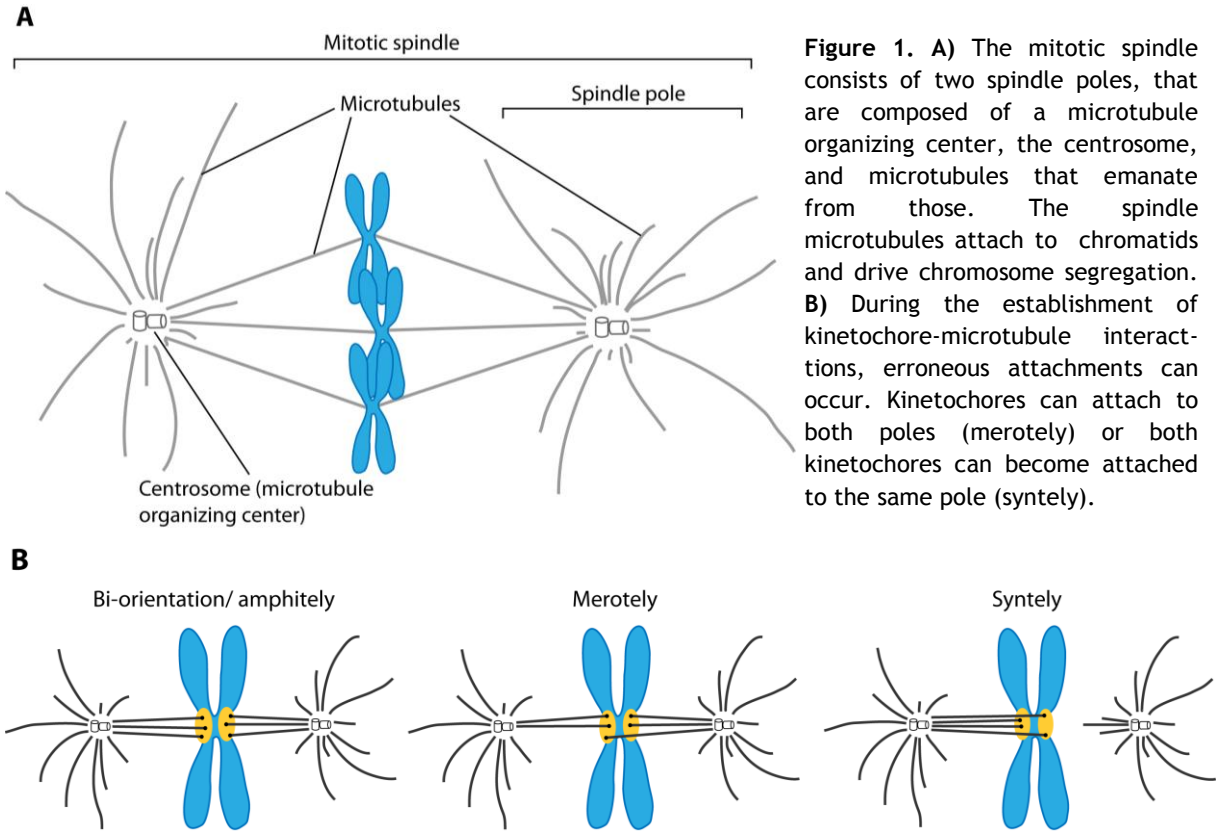

Figure 1. A) The mitotic spindle consists of two spindle poles, that are composed of a microtubule organizing center, the centrosome, and microtubules that emanate from those. The spindle microtubules attach to chromatids and drive chromosome segregation. **B)** During the establishment of kinetochore-microtubule interact-tions, erroneous attachments can occur. Kinetochores can attach to both poles (merotely) or both kinetochores can become attached to the same pole (syntely).

poles instead of one and can lead to lagging chromosomes, that are not always successfully distributed to the correct daughter cell (Cimini *et al.*, 2001) (Figure 1B). In another kind of erroneous kinetochore-microtubule orientation, syntely, both sister chromatids are bound to the same spindle pole (Figure 1B). Syntely could, if not corrected, also lead to unequal chromosome segregation. To prevent errors in the distribution of the genetic information between daughter cells, the spindle assembly checkpoint (SAC) and correction processes are active during mitosis. The main function of the SAC is to postpone chromosome segregation until all chromatids are bound to microtubules. Mal-orientations are thus not detected by SAC, because they deliver bound chromatids. Cells, therefore, are able to prevent and to correct erroneous attachments. In this thesis, I aim at summarizing the current knowledge about error prevention and correction and attempt to combine this information into a proper model.

Prevention of erroneous attachments

Kinetochore-microtubule attachment

To understand how erroneous attachments can occur and how they are prevented and corrected, it is essential to understand how attachment of kinetochores to microtubules is established. Initial microtubule capture by kinetochores has been suggested to be a result of random "search-and-capture" (Kirschner and Mitchison, 1986; O'Connell and Khodjakov, 2007). Microtubules emanate from the spindle poles in all directions, growing and shrinking at their plus ends, until they meet a kinetochore and are stabilized. In this way, chromosomes are mono-oriented, bound to one pole, at first, but become attached to both poles eventually (reviewed in Walczak and Heald, 2008). Interestingly, mathematical modeling has shown that if microtubule capture and spindle assembly would only be dependent on stochastic events, as is suggested by the "search-and-capture" model, these processes would take much more time than they do *in vivo*. Thus, additional mechanisms that accelerate microtubule capture must be active during mitosis (Wollman *et al.*, 2005). One of these mechanisms is the Ran-GTP gradient, that exists around centromeres and is essential for spindle assembly. This gradient is a favorable environment for microtubule polymerization and stabilization, therefore providing spatial information that contributes to the directed growth of microtubules towards the kinetochores (Clarke and Zhang, 2008; Kalab *et al.*, 2006; Walczak and Heald, 2008; Wilde *et al.*, 2001). Additionally, it has been suggested that kinetochores contribute to acceleration of kinetochore capture by nucleating microtubules, independent of the centrosomes, near or within themselves (Maiato *et al.*, 2004; Witt *et al.*, 1980). These microtubules then use kinetochore-bound microtubules to move towards one of the spindle poles and integrate in it, allowing that kinetochore to establish mono-oriented attachment (Maiato *et al.*, 2004). The relative contribution of this process to kinetochore-microtubule attachment, as well as the molecules that play a role in this process remain unknown. In conclusion, the "search-and-capture" mechanism is responsible for the initial kinetochore-microtubule attachments. The Ran-GTP gradient and centrosome-independent microtubule polymerization act in addition to the "search-and-capture" mechanism to moderate its stochastic character. These mechanisms together do not, however, provide information that enables kinetochores and microtubules to distinguish between correct and erroneous attachments, indicating that additional mechanisms fulfill this function.

Sister kinetochore geometry

To increase the chance of proper bi-orientation, sister kinetochores are oriented back-to-back, referring to a state of orientation wherein an unattached kinetochore faces automatically to one pole when its sister becomes attached to the other pole. Interestingly, it has been shown that the orientation of sister kinetochores relative to each other is dependent on the state of attachment of those kinetochores to microtubules: syntelic attachments lead to sister kinetochores becoming juxtaposed to one side of the paired sister chromatids, indicating that sister kinetochores are connected to each other in a flexible manner (Lončarek *et al.*, 2007). Severing those syntelic

attachments, however, did not revert sister kinetochore juxtaposing, showing that the connections between kinetochores are not elastic in nature. In addition, tethering chromosome pairs near the centromeres during meiosis I in yeast deficient of SAC promotes successful division. This observation shows that constraining kinetochores of homologous chromosomes to face opposite sides is sufficient to prevent mal-orientations when other mechanisms that promote successful division during meiosis are defective (Lacefield and Murray, 2007). Conservation of back-to-back geometry is thus important for proper chromosome segregation.

The composition of heterochromatin has been suggested to have a major role in the geometry of kinetochores. At the centromeric region of the heterochromatin, the nucleosomes are divergent from those at the chromosome arms. The centromere region contains the histone H3-variant CENP-A, that has been shown to be required for proper chromosome segregation and embryonic survival in mice, and kinetochore assembly in *Drosophila* and human cells (Blower and Karpen, 2001; Howman *et al.*, 2000; Van Hooser *et al.*, 2001). A possible explanation for kinetochore geometry was provided by the observation that CENP-A-containing nucleosomes alternate with H3-containing nucleosomes (Blower *et al.*, 2002). It has been suggested that such arrangement of nucleosomes can create a CENP-A-surface facing the pole by accurate looping or solenoid organization of the chromatin (Blower *et al.*, 2002). The particular localization of CENP-A presumably contributes to proper geometry of kinetochores. This model, however, remains speculative. Other possible contributors to proper kinetochore geometry are the related cohesin and condesin complexes (Hirano, 2002). Both complexes are explicitly enriched at the chromatin regions surrounding the centromere (Hagstrom *et al.*, 2002; Ono *et al.*, 2003; Tomonaga *et al.*, 2000) and localize specifically at the inner side of the sister chromatids at the chromatid interface. Cohesin and condesin are thus elements of the chromatin structure that underlies kinetochores. Correct recruitment of cohesin to the pericentromeric regions is crucial for proper chromosome segregation (Ekwall *et al.*, 1995; Nonaka *et al.*, 2002; Tomonaga *et al.*, 2000) and is dependent on the association of cohesin with Heterochromatin Protein 1, an essential centromere protein (Bernard *et al.*, 2001; Nonaka *et al.*, 2002). Interestingly, depletion of cohesin leads to the loss of bi-orientation in budding yeast and vertebrate cells and it has been suggested that this might be due to the role of cohesin in joining sister kinetochores back-to-back (Sonoda *et al.*, 2001; Tanaka *et al.*, 2000). Similarly, depletion of condesin leads to loss of kinetochore geometry: kinetochores are disorganized or move closer to each other (Hagstrom *et al.*, 2002; Ono *et al.*, 2004). Together, these indicate that CENP-A ad the cohesion and condesin complex could be involved in ensuring back-to-back kinetochore geometry and therefore promoting proper chromosome segregation.

Despite all this evidence, the role for kinetochore geometry in preventing mal-orientations is still a controversial issue. It has been suggested that kinetochore geometry does not necessarily lead to bi-orientation (Cimini *et al.*, 2003). Additionally, it has been shown that lack of back-to-back geometry does not prevent bi-orientation: kinetochores that have lost their sister can bind stably to microtubules and congress to the metaphase plate (Dewar *et al.*, 2004; Khodjakov *et al.*, 1997). Taken together, kinetochore geometry seems not to be essential for bi-orientation, but is

presumably facilitating the whole process. However, when erroneous connection are made, geometry is not sufficient to correct these. Therefore, additional mechanisms are active that will be discussed in the next chapter.

Spindle geometry

Not only kinetochore geometry, but spindle geometry as well seems to contribute to the optimization of bi-orientation. Properly segregated spindle poles make kinetochore binding to opposite poles easier, especially in combination with kinetochore back-to-back geometry. This was discovered when *sgo1* budding yeast mutants were found to behave remarkably after treatment with microtubule depolymerization drugs. Microtubule depolymerization usually leads to mitotic delay as a consequence of several defects in spindle assembly. After removal of the microtubule depolymerization drugs, however, wildtype cells are able to fulfill chromosome segregation correctly. *Sgo1* mutants do not restore chromosome segregation after drug removal and die (Indjeian *et al.*, 2005). Further investigation of the response of the *sgo1* mutants showed that cell survival of the mutants depends on the moment of drug removal. Chromosome segregation and cell survival were accomplished only when microtubule dynamics were restored after proper spindle pole segregation (Indjeian and Murray 2007), indicating that spindle geometry is essential for normal chromosome segregation. Thus, spindle geometry promotes proper kinetochore orientation.

Additionally, it is possible that kinetochore back-to-back orientation is dependent on spindle geometry. It has been shown that positioning of sister kinetochores, juxtaposed in syntelic orientation or back-to-back in amphitely, requires external forces that set kinetochores in motion (Lončarek *et al.*, 2007). Because the spindle microtubules presumably are responsible for this, it is arguable that proper spindle pole segregation promotes kinetochore back-to-back orientation and thus proper bi-orientation and chromosome segregation.

Correction mechanisms of erroneous attachments

Once erroneous connections between kinetochores and the mitotic spindle are made it is essential for the achievement of bi-orientation to convert those attachments into proper ones. The simplest way to achieve this, is to remove the incorrectly bound microtubules from the kinetochore. By doing so, availability of the kinetochore is restored and new and, hopefully, correct microtubule attachments can be made. Aurora B kinase has been shown to be the major regulator of kinetochore-microtubule attachments. In this chapter, I will discuss the mechanisms of error correction by Aurora B.

Aurora B regulates kinetochore-microtubule attachments

Aurora B kinase is a component of the conserved Chromosomal Passenger Complex (CPC), that acts in kinetochore maturation, spindle midzone formation, and several other processes that take place during mitosis (Ruchaud *et al.*, 2007). Inhibition of Aurora B leads to persistent syntelic and merotelic orientations, indicating that Aurora B is involved in the correction of erroneous attachments (Cimini *et al.*, 2006; Hauf *et al.*, 2003; Lampson *et al.*, 2004). Aurora B specifically regulates kinetochore-microtubule connections by regulating the activity of the KMN network, Dam1 and MCAK. Below, I will further clarify the function of Aurora B in kinetochore-microtubule attachments.

The KMN network

Aurora B phosphorylates multiple components of the KMN network, that forms the kinetochore-microtubule interface (Cheeseman *et al.*, 2006; Ciferri *et al.*, 2008; DeLuca *et al.*, 2006; Wei *et al.*, 2007, Welburn *et al.*, 2010). The KMN network consists of three protein complexes, the KNL1, Mis12 and Ndc80 complex, that together form the flexible outer kinetochore plate to which microtubules can attach (Dong *et al.*, 2007; McEwen and Dong, 2010). Additionally, phosphorylation of the KMN network by Aurora B has been proposed to be required for kinetochore assembly (Emanuele *et al.*, 2008), although this proposal has been doubted, because the effect of Aurora B on kinetochore assembly has been shown to be dispensable (Welburn *et al.*, 2010).

The best studied Aurora B substrate in the KMN network is Ndc80 (Cheeseman *et al.*, 2006; Ciferri *et al.*, 2008; DeLuca *et al.*, 2006). Ndc80 forms together with Nuf2, Spc24 and Spc25 the Ndc80 complex, which is highly conserved from yeast to vertebrates (Kline-Smith *et al.*, 2005; Maiato *et al.*, 2004). All subunits of the complex contain a globular domain and long coiled-coil domains, through which they interact with each other (Ciferri *et al.*, 2005; Wei *et al.*, 2005). Ndc80 connects kinetochores to microtubules by interaction of its N-terminal globular domain to the microtubule lattice (Cheeseman *et al.*, 2006). Phosphorylation of this domain by Aurora B leads to disruption of the interactions between Ndc80 and the microtubule and therefore inhibits the affinity of Ndc80 for microtubules (Cheeseman *et al.*, 2006; DeLuca *et al.*, 2006; Guimaraes *et al.*, 2008; Miller *et al.*, 2008). Surprisingly, inhibition of the phosphorylation of the Ndc80 N-terminus, and thus prevention

of establishment of weak kinetochore-microtubule interactions, led to robust but erroneous kinetochore-microtubule attachments (DeLuca *et al.*, 2006, Miller *et al.*, 2008). This shows that kinetochore-microtubule attachments that are too solid are disadvantageous for bi-orientation. In conclusion, Aurora B is able to regulate kinetochore-microtubule interactions by weakening them through phosphorylation of Ndc80. In addition, phosphorylation of Ndc80 is essential for proper bi-orientation.

Other kinetochore substrates that are phosphorylated by Aurora B and are involved in kinetochore-microtubule interactions are Dsn1, a subunit of the Mis12 complex, and KNL1 N-terminus (Welburn *et al.*, 2010). Of these two substrates, only KNL1 binds directly to microtubules; the Mis12 complex links the KNL1 and Ndc80 to the inner kinetochore and does not associate with microtubules (Cheeseman *et al.*, 2006; Petrovic *et al.*, 2010). Therefore, Dsn1 phosphorylation does not weaken kinetochore-microtubule interactions directly, but its phosphorylation adds to the weakening effect of KNL1 or Ndc80 phosphorylation (Welburn *et al.*, 2010). Thus, phosphorylation of different KMN substrates by Aurora B leads to gradually changing microtubule-binding activity of the kinetochore (Welburn *et al.*, 2010), showing that interactions between kinetochores and microtubules are not an on-or-off state, but can be fine-tuned.

Dam1

Yeast have, in addition to the KMN network, another microtubule-interacting complex whose function is regulated by Aurora B phosphorylation: the Dam1 complex. This complex assembles at the microtubule tips in a ring (Miranda *et al.*, 2005; Westermann *et al.*, 2005) and has been shown to associate with components of the kinetochore (Cheeseman *et al.*, 2001; Janke *et al.*, 2002; Tien *et al.*, 2010). Dam1 is, thus, involved in kinetochore-microtubule interactions in yeast. In fact, Dam1 is essential for kinetochore attachment to depolymerizing microtubules, referred to as the plus-end tracking activity of the kinetochore (Lampert *et al.*, 2010). Depletion of Dam1 leads to defects in bi-orientation, showing that Dam1 plays an important role in the establishment of correct attachments during mitosis (Cheeseman *et al.*, 2002; DeLuca *et al.*, 2006). Phosphorylation of Dam1 at the C-terminus by Aurora B (Cheeseman *et al.*, 2002; Wang *et al.*, 2007) leads to a weakend interaction between Dam1 and Ndc80 and thus destabilization of kinetochore-microtubule associations (Shang *et al.*, 2003; Tien *et al.*, 2010), indicating that Aurora B regulates kinetochore-microtubule interactions by phosphorylating Dam1. Furthermore, depletion of PP1, the phosphatase that opposes Aurora B (Francisco *et al.*, 1994), in combination with phospho-mimicking Dam1 leads to synthetic lethality in yeast (Cheeseman *et al.*, 2002; Shang *et al.*, 2003). Additionally, both phosphorylation defective as consecutive phosphorylation Dam1 leads to dramatic chromosome mis-segregations. These data imply that it is the turnover of Dam1, from a phosphorylated to an unphosphorylated state, that is important for error correction. Although Dam1 fulfills an important function in yeast, its homologue is not been found in multicellular organisms. However, the Ska1 complex has been suggested to fulfill the same function as Dam1, although homology is lacking (Hanisch *et al.*, 2006; Welburn *et al.*, 2009) and no regulation of the Ska1 complex by Aurora B has

yet been shown. Taken together, these data show that Aurora B regulates interactions between kinetochores and microtubules by phosphorylating multiple substrates involved in that interaction, for example Dam1.

MCAK

A third well-studied substrate of Aurora B involved in error correction is the microtubule depolymerizing protein MCAK (Bakhoum *et al.*, 2009; Illingworth *et al.*, 2010; Kline-Smith *et al.*, 2004; Wordeman *et al.*, 2007). Upon phosphorylation by Aurora B, MCAKs microtubule depolymerizing activity is inhibited and MCAK is delivered at the centromeres (Andrews *et al.*, 2004; Lampson *et al.*, 2004; Lan *et al.*, 2004). Interestingly, MCAK is enriched at merotelic sites in an Aurora B-dependent manner (Knowlton *et al.*, 2006, Lampson *et al.*, 2004), underscoring the role of MCAK in error correction. Additionally, it has been observed that Aurora B corrects syntelic attachments by promoting disassembly of microtubules, leading to the movement of these chromosomes towards the pole they are attached to (Lampson *et al.*, 2004). This could be due to the activity of MCAK, although for chromosomes to move along with depolymerizing microtubules, stable attachments are required that lack in syntelic attachments (see below). These studies indicate that Aurora B could be involved in error correction by regulating the microtubule depolymerizing activity of MCAK. Thus Aurora B regulates the interaction between sister chromatid kinetochore and spindle pole microtubules via the phosphorylation of multiple substrates that regulate this association.

Although it is widely accepted that MCAK plays an important role in error correction, it still remains unclear how MCAK exactly contributes to this process. Microtubule depolymerization can only lead to loss of kinetochore-microtubule attachments when these attachments are weak. This means that for MCAK to act in error correction, the KMN network has to be phosphorylated to weaken kinetochore-microtubule interactions, while MCAK should remain unphosphorylated. However, MCAK has been shown to localize at the kinetochores and centromeres (Andrews *et al.*, 2004), suggesting that MCAKs phosphorylated state always resembles that of the KMN network. Therefore, error correction by MCAK is possibly not dependent on Aurora B phosphorylation alone. Additional mechanisms are active that allow MCAK to be unphosphorylated and to correct misattached microtubules. One of these mechanisms could for example be the association of MCAK to ICIS. ICIS is a protein that localizes to the surface of the inner centromere by interacting with Aurora B and other subunits of the Aurora B complex (Ohi *et al.*, 2003). Association between MCAK and ICIS has been suggested to promote MCAK activity (Ohi *et al.*, 2003), although it remains unclear how MCAK can gain activity while being localized in the proximity of Aurora B. Correction of misattached microtubules could then take place, because the orientation of these microtubules enables them to proximate ICIS enriched locations and be depolymerized by active MCAK. This mode of correction would not interfere with MCAK and the KMN network having the same state of phosphorylation at the kinetochores. Another explanation for the controversy in the MCAK-dependent error correction mechanism, could be that other microtubule depolymerases are also involved in error correction.

Kif2a and Op18 are depolymerases that have been shown to be dependent on Aurora B during mitosis for their activity (Desai *et al.*, 1999, Gadea and Ruderman, 2006; Ohi *et al.*, 2007). In addition, Kif2a localizes to the spindle poles and centromeres and depletion of Kif2a leads to the collapse of the mitotic spindle inducing mistakes in chromosome segregation (Ganem and Compton, 2004; Knowlton *et al.*, 2009; Manning *et al.*, 2007; Zhu *et al.*, 2005). Furthermore, similar to what has been suggested for MCAK, Kif2a activity is promoted when it interacts with ICIS (Knowlton *et al.*, 2009). Op18 is found to be highly upregulated in breast tumors (Curmi *et al.*, 2000) and is associated with mistakes in mitosis and mis-aligned chromosomes (Andersen, 2000). Thus, Kif2a and Op18 could act in parallel to MCAK in error correction or contribute to MCAKs activity in the removal of incorrect attachments. A third possible explanation is that the flexible kinetochore is not as a whole phosphorylated or unphosphorylated and that its phosphorylation state depends on the microtubule attachments. In conclusion, it is unclear how MCAK exactly acts in error correction. It is assumed that MCAK depolymerizes incorrectly bound microtubules. However, it is impossible to understand how MCAK at the kinetochores can be active, thus unphosphorylated, while at the same time the Ndc80 complex is phosphorylated to allow detachment of incorrectly bound microtubules. Therefore, additional mechanisms possibly contribute to MCAK activity in error correction.
Altogether, these data show that Aurora B regulates kinetochore-microtubule interactions by phosphorylating the KMN network, MCAK and Dam1. This regulation contributes to the establishment of bi-orientation. However, it is unknown how exactly the phosphor-regulation of the different substrates are related to each other in the correction of erroneous attachments.

The role of tension in correction of erroneous attachments

It is remarkable that Aurora B only removes erroneous connection, while leaving correct associations intact. In what way is Aurora B then capable of distinguishing erroneous attachments from correct ones? Or in other words: how do bi-oriented kinetochores escape destabilization by Aurora B, while mal-orientation do not?
The major difference between correct and incorrect attachments is the tension across sister kinetochores that is generated by bound microtubules. In case of bi-orientation, microtubules apply pulling forces on kinetochores and centromeres (Tanaka *et al.*, 2000). These forces lead to the creation of tension across kinetochores, because cohesion between the kinetochores is working as a counteracting force on the pulling forces. It has been suggested that any kind of cohesion between sister kinetochores is sufficient to fulfill this function (Dewar *et al.*, 2004; Vagnarelli *et al.*, 2004), although in cells that role is assigned to cohesins (Sonoda *et al.*, 2001; Tanaka *et al.*, 2000). Thus, in bi-oriented cells, pulling forces from opposite sides on two structures that are linked, creates tension. If pulling, however, comes from the same side as is the case in syntely, sister kinetochores are barely pulled away from each other and tension across the sister kinetochores is lacking (Figure 2A). Correct and incorrect attachments thus differ in the amount of tension that is generated at the kinetochores.

As it turns out, Aurora B-mediated phosphorylation of the kinetochores is dependent on tension (Nicklas *et al.*, 1998); in the absence of tension, kinetochores are phosphorylated and microtubule turnover is promoted, resulting in the dissociation of incorrect kinetochore-microtubule connections (Cimini *et al.*, 2006; Dewar *et al.*, 2004; King and Nicklas, 2000; Pinsky *et al.*, 2006; Tanaka *et al.*, 2002). These observations suggest that Aurora B reacts on tension by either being dependent on the absence of tension for its activation or by being differentially localized relative to its substrates in the presence of tension (Tanaka *et al.*, 2002). The latter mechanism has been shown to be true; Aurora B is active at both correct and incorrect oriented kinetochores and whether Aurora B phosphorylates its substrates depends on the spatial separation of substrates and kinase. Thus, when tension is applied, for example as a consequence of bi-orientation, the kinetochores are pulled away from the inner centromeric Aurora B activity (Figure 2A). In addition, stabilization of bi-oriented kinetochore-microtubule attachments is impaired when Aurora B is artificially placed closer to the kinetochores (Liu *et al.*, 2009), showing that phosphorylation of the kinetochore substrates, for example Dam1 (Keating *et al.*, 2009), and microtubule stabilization are dependent on spatial separation from the sphere of influence of Aurora B. Creating tension and thus spatial separation across sister kinetochores is, in turn, dependent on correct, bipolar kinetochore-microtubule attachments. For example, loss of the N-terminal tail of Ndc80 leads to loss of tension across kinetochores (Miller *et al.*, 2008).

Interestingly, it has been suggested that MCAK is also implicated in the generation of tension across sister kinetochores. However, there is controversy about whether MCAK increases or decreases kinetochore tension (Kline-Smith *et al.*, 2004; Wordeman *et al.*, 2007). In case of bi-orientation, and thus stable kinetochore-microtubule interactions, MCAK stimulates microtubule depolymerization, causing the microtubules to practice force on the kinetochores and pulling them apart. However, promotion of microtubule turnover at the kinetochores could also lead to loss of kinetochore-microtubule attachments and thus loss of tension. Additionally, MCAK phosphorylation by Aurora B is a perfect example of how spatial regulation of the activity of MCAK is correlated to the generation of tension across kinetochores. MCAK has been shown to localize at both the kinetochores and centromeres (Andrews *et al.*, 2004). These pools of MCAK are, however, divergent: phosphorylated MCAK localizes in an Aurora B-dependent manner at the centromeres, while unphosphorylated MCAK localizes at kinetochores (Andrews *et al.*, 2004). Another study showed that the pool of MCAK that localizes at the inner centromere, co-localizes with Aurora B, while the rest of MCAK that localizes to the kinetochores is not in the proximity of Aurora B, leading to the dephosphorylation of MCAK (Lan *et al.*, 2004). These results show that MCAK is under spatial regulation of Aurora B. MCAK is phosphorylated, and inactivated, when it is in the proximity of Aurora B at the inner centromere and unphosphorylated, and thus active, when it is at the kinetochores. Surprisingly, ICIS has been suggested to act as a receptor for either the phosphorylated or the unphosphorylated form of MCAK (Andrews *et al.*, 2004). Furthermore these results show that MCAK cannot be the initial force generator, as it is only active after kinetochores

are separated and thus force is generated. Therefore, it is reasonable that MCAK contributes to the process of force generation, in addition to its function in error correction.

The tension-dependent function of Aurora B in error correction raises a few questions. It is, for example, unclear why mono-oriented chromosomes persist and are the first step towards bi-orientation (Kops *et al.*, 2010; Tanaka *et al.*, 2005). Initially, kinetochores are bound to the microtubule lattice, along which they are transported towards the poles in a dynein-dependent manner. Along the way, these lateral attachments are converted into end-on attachments in an unknown mechanisms (Kops *et al.*, 2010; Tanaka *et al.*, 2005). During the first attachments, no tension is created along the kinetochore, although the kinetochore-microtubule attachments are stable enough to transport chromosomes via the microtubule towards one of the poles. The absence of error correction in these attachments, is not caused by inactivity of Aurora B-dependent mechanisms at the moment these attachments are made. Mal-orientations are namely found more frequently during pro-metaphase than during metaphase and anaphase (Hauf *et al.*, 2003), indicating that correction mechanisms are already active when initial kinetochore-microtubule contacts are made. Lack of correction may be a consequence of the additional mechanisms that protect these initial contacts. Another explanation could be that these attachments are lost as a consequence of error correction, but are then reformed quickly, because they are easily established. Interestingly, it was shown recently that the composition of the kinetochores changes during mitosis in an Aurora B activity-dependent manner. This indicates that the kinetochore properties can change and could explain why error correction is not active during the establishment of initial contacts (Schmidt *et al.*, 2010).

Other questions about the role of tension in kinetochore-microtubule associations arise, when congression takes place. During congression, mono-oriented chromosomes move towards the equator via attachment of the unbound kinetochore to the lattice of microtubules. It is unclear whether these kind of attachments produce tension across kinetochores. If so, how is the stable attachment that follows from tension, destabilized to allow bi-orientation? If not, how would the chromosomes congress? Perhaps in this situation Aurora B is specifically prevented from phosphorylating the kinetochores, to enable congression. For example, by being a slow kinase, allowing kinetochores to bind stably to the microtubule lattice long enough to move to the cell equator.

Another important question about the role of tension in error correction is: when does correction stop? Why are the kinetochore-microtubule attachments not removed after chromosome segregation, when no tension is generated at the kinetochores? This might be due to the relocation of Aurora B from kinetochores to the spindle upon anaphase onset. Interestingly, this relocation is dependent on dephosphorylation of subunits of the Aurora B complex, that, in turn, is dependent on the absence of tension across centromeres (Mirchenko and Uhlmann, 2010; Vázquez-Novelle and Petronczki, 2010). In fact, inhibition of Aurora B relocation leads to re-orientation of kinetochores at the metaphase plate (Parry *et al.*, 2003).

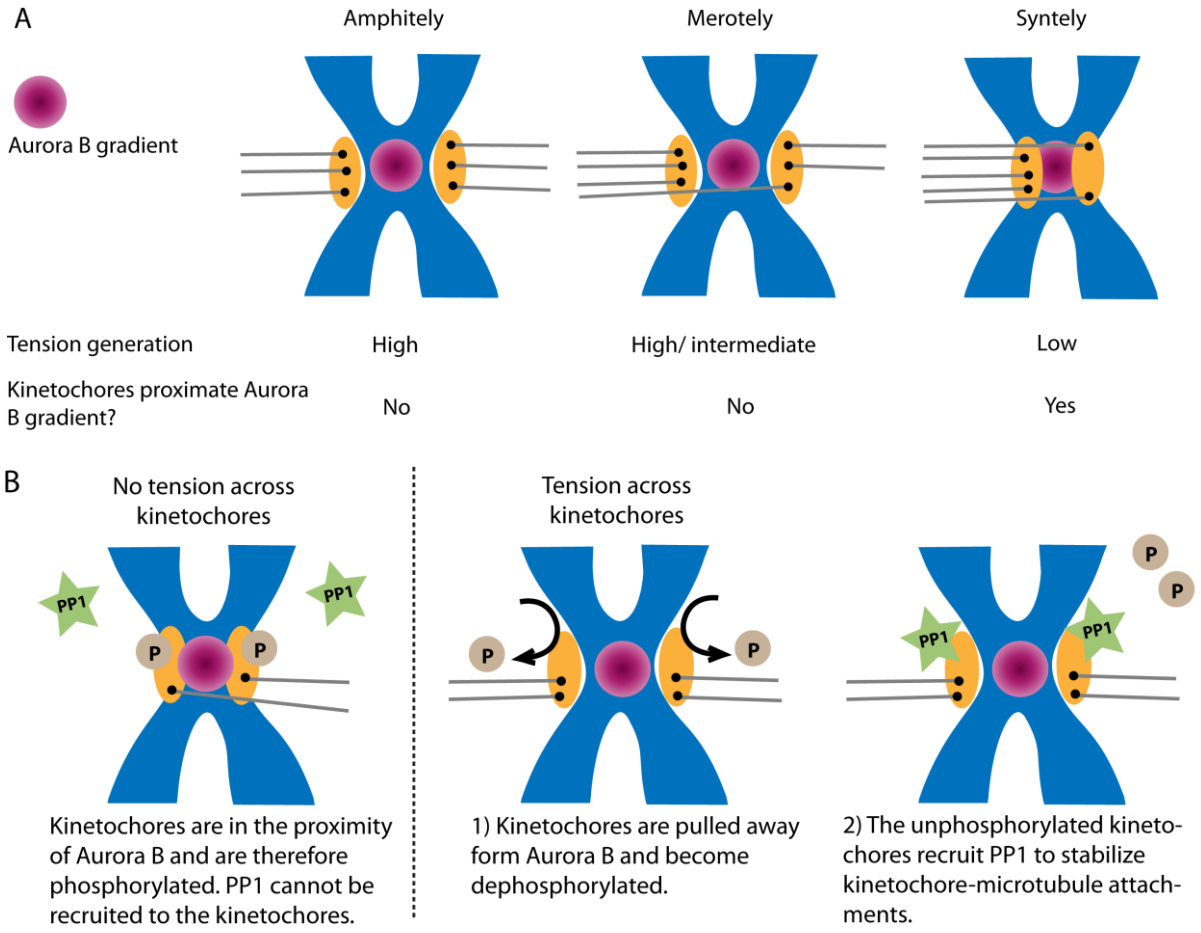

Figure 2. A) When merotelic or syntelic attachments are made, the tension across kinetochores is not optimal and kinetochores remain in the proximity of the Aurora B gradient. As a consequence, kinetochores are phosphorylated and kinetochore-microtubule interactions are lost. **B)** PP1 binds to the kinetochores when KNL1 is not phosphorylated by Aurora B. There, PP1 dephosphorylated the kinetochores and kinetochore-microtubule (KT-MT) interactions are stabilized. However, the kinetochores are phosphorylated once again, when the bound microtubules are not able to generated tension across kinetochores, for example in the case of syntely.

In conclusion, Aurora B phosphorylation of substrates involved in kinetochore-microtubule interaction depends on the amount of tension applied on the kinetochores. Only when microtubules are bi-oriented, maximum tension is generated across sister kinetochores and will lead to the spatial separation of the substrates from Aurora B (Figure 2A). This will then lead to stabilization of the association between kinetochores and microtubules. As depicted before, syntelic orientations generate less or no tension at all across kinetochores, leading to phosphorylation of the kinetochores and detachment of the erroneous attachments (Figure 2A). Interestingly, merotely does not, or barely, lead to decrease of kinetochore stretch; only one kinetochore-bound microtubule from the incorrect pole is enough to cause lagging chromosomes, without affecting the kinetochore stretch (Figure 2A). Thus, merotelically oriented kinetochores do not reach the proximity of Aurora B and remain unphosphorylated. However, merotelic kinetochores do show intra kinetochore stretch (Courtheoux *et al.*, 2009), because they are pulled to opposing sides by their bound microtubules. Thus, syntely and merotely both have a different mode of tension generation

at the kinetochore compared to amphitely. Probably, this difference leads to the correction of merotely and syntely.

PP1 antagonizes Aurora B function

If tension causes inhibition of phosphorylation by Aurora B, it follows that substrates need to be dephosphorylated before tension can be established. Consequently, Aurora B function is antagonized by PP1 (Hsu *et al.*, 2000; Liu *et al.*, 2010; Sasoon *et al.*, 1999). Additionally, PP1 not only antagonizes Aurora B function, but also inhibits its activity (Murnion *et al.*, 2001). Furthermore, PP1 is localized to the outer kinetochores and pericentromeric chromatin during metaphase (Andreassen *et al.*, 1998; Trinke-Mulcahy et al 2003), and therefore fits perfectly in the role of Aurora B antagonizor during establishment of kinetochore-microtubule attachments. PP1 recruitment to the outer kinetochore is mediated by among others KNL1 and is essential for stable kinetochore-microtubule attachments and dephosphorylation of the kinetochore (Liu *et al.*, 2010). Surprisingly, KNL1 phosphorylation by Aurora B inhibits recruitment of PP1 to the kinetochore (Liu *et al.*, 2010), meaning that kinetochores have to be removed from the sphere of the Aurora B gradient before they can be dephosphorylated (Figure 2B). But for kinetochores to be spatially removed from Aurora B kinase activity, stable microtubule attachments are essential. Contradictory, these can only be made when kinetochores are dephosphorylated. Or in other words: if PP1 is needed to dephosphorylate the KMN network, how can it bind to the network in the first place? It could be that other phosphatases are active in the dephosphorylation of the KMN network, although other antagonizers of Aurora B have not been reported yet. Another explanation could be that PP1 localizes to the kinetochores in low concentrations even when the KMN network is still phosphorylated, suggesting that kinetochores are dephosphorylated all the time to allow the generation of tension across kinetochores. In addition, it has been shown that unphosphorylated kinetochore motor CENP-E, which is a substrate of Aurora B, has the ability to recruit PP1 to the kinetochores (Kim *et al.*, 2010). It remain unclear, however, why this protein remains unphosphorylated before bi-orientation, and thus the generation of tension, is achieved. A third explanation for these contradictory mechanism could be that Aurora B is a slow kinase. This would mean that kinetochores that proximate the Aurora B gradient are not directly phosphorylated, allowing the kinetochores to attach to microtubules firmly to spatial remove themselves and to recruit PP1. Together these results indicate that PP1 acts in kinetochore bi-orientation by opposing Aurora B function. Presumably, PP1 is specifically recruited to the kinetochores by players of kinetochore bi-orientation. Exact details of this mechanism remain unknown.

Model for correction of erroneous attachments

To establish proper distribution of the genetic information between daughter cells, bi-orientation of chromosomes has to be achieved. For this, it is essential that erroneous kinetochore-microtubule attachments are corrected. Correction of both syntelic and merotelic attachments is assumed to be a result of loss of the incorrect kinetochore-microtubule attachment, controlled by Aurora B. The mechanisms underlying the correction of these attachments is, however, slightly different.

Syntely is suggested to be corrected as follows: upon syntelic attachment, tension across sister kinetochores is lost, leading to an encounter between the KMN network and the sphere of the Aurora B gradient. As a consequence, kinetochore-microtubule interactions are lost, making kinetochores free for new attempts to establish bi-orientation. This model, however, leaves room for improvement. For example, syntelically oriented chromatids have been observed to move towards the pole they are attached to before being corrected. This means that the KMN network in syntelic attachments is bound stably enough to microtubules to mediate the transport of the chromosomes towards the pole they are attached to. Thus, kinetochore-microtubule attachments are not lost immediately after syntely is established. The KMN network is rather not directly or only partly phosphorylated until chromatids reach the pole they are attached to, where the incorrect attachments are lost. Previously it was shown that syntelic attachments are converted to monotelic ones, which then congress to the metaphase plate via a CENP-E dependent mechanism (Kapoor *et al.,* 2006). However, there are no indications that kinetochore-microtubule detachment on syntelic oriented chromosomes would have a bias towards one of the kinetochores. Therefore, both kinetochores have equal chance of being detached from spindle microtubules and probably are detached simultaneously. Nevertheless, new attachments and congression can be easily achieved because multiple mechanisms are active to establish kinetochore-microtubule interactions. The freed kinetochores can, for example, associate with new microtubules from the pole they are in the proximity of or they can associate to the lattice of other microtubules. Additionally, kinetochores could assembles their own microtubules and use those to achieve bi-orientation. The best explanation for correction of syntelic attachments is, however, that Aurora B is a slow kinase. If Aurora B does not immediately phosphorylate every substrate that comes near it, that would mean that syntelically oriented kinetochores are initially stable enough to mediate the initial poleward chromatid transport. Additionally, the slow Aurora B kinase explains how MCAK could contribute to the poleward movement of syntelically oriented chromatids. If Aurora B would immediately phosphorylate MCAK upon syntelic orientation, MCAK would be inactive and would not be able to provide for the poleward movement. Except if the collapse of the kinetochore and centromere as a consequence of syntely, would in an encounter between MCAK and phosphatase-rich regions of the centromere. In that case, MCAK would be dephosphorylated and active. This remains a rather speculative hypothesis. A more acceptable explanation could be that other depolymerases, for example Kif2a or Op18, could fulfill the microtubule depolymerizing role of MCAK during syntely. If Aurora B would not phosphorylate MCAK immediately after the collapse of the kinetochores and

centromere, opportunity would be created for MCAK to depolymerize bound microtubules and to move syntelically oriented chromatids towards one pole. In this case, eventual correction of syntely would not be due to MCAK activity, as that is inhibited by the time the chromatids reach the pole, but would be dependent on other mechanisms, for example only loss of attachment between the KMN network and microtubules. Interestingly, the slow Aurora B kinase would also add to efficient achievement of bi-orientation. Kinetochores would have time to mono-orient at first, to become bi-oriented eventually.

Merotelic orientation are corrected differently, as they do not lead to decreased tension across kinetochores. They do, however, create intra kinetochore stretch. In addition, merotely has been shown to lead to correction or microtubule disruption (Courtheoux *et al.*, 2009), reflecting that merotely can be corrected without necessarily disassembling all kinetochore-microtubule attachments of the incorrect attached kinetochore. When merotelic attachments are made, the incorrect microtubules on the kinetochore, the microtubules on the kinetochore that attach the kinetochore to the wrong pole, pull Aurora B substrates and MCAK in the vicinity of the centromere, leading to weakening of those attachments and activation of MCAK (Figure 3A). Meanwhile, the part of the MCAK pool that is located at correctly bound segments of the kinetochore, is pulled away from Aurora B. This causes local activation of MCAK and therefore the generation of tension, on its turn leading to increased stabilization of those attachments. Together, these mechanisms lead to detachment of the incorrectly bound microtubules and stabilization of the correctly bound microtubules on the same kinetochore. Different processes taking place at the same kinetochore is possible, because of the flexible nature of the outer kinetochore.

In addition, I propose that the ratio of microtubules from the correct pole relative to the amount of microtubules from the incorrect pole defines whether all attachments on a merotelic oriented chromosome are lost or not. When the largest part of the kinetochore is occupied by kinetochores from the incorrect pole, the pulling forces could be so high that almost all segments of the kinetochore will be pulled close to the Aurora B gradient, leading to disruption of the attachments. This would then lead to weakening of both the correct and incorrect kinetochore-microtubule attachments. However, the correct attachments are not necessarily lost, because kinetochore-microtubule attachments are not an on-or-off state, but more gradually regulated (Welburn *et al.*, 2010). Additionally, it could be that the kinetochores become more or less juxtaposed, as happens in syntelic orientations, as a consequence of the high pulling forces from the incorrect pole (Figure 3B). Then these merotelic attachments could be corrected similar to syntelic ones. When most microtubules are bound to the correct pole, segments of the kinetochore that are attached to microtubules from the incorrect pole, are placed more distantly from Aurora B and become stabilized. Nevertheless, this does not lead to lagging chromosomes, as a consequence of the pulling forces that are higher at the side of the correct pole (Salmon *et al.*, 2005) (Figure 3A). Additionally, this model explains why microtubule ratios of one, the kinetochore is bound to equal amounts of microtubules from both poles, could lead to lagging chromosomes. These kinetochores balance at the border of having stable and unstable attachments (Figure 3C). Pulling from the correct pole is

just enough to stabilize the attachments from the incorrect pole. This will then lead to the generation of counterforce, pulling the kinetochore closer to Aurora B just enough to give the forces from the correct pole the upper hand. Eventually, this could cause the absence of correction of these mal-orientations and lead to lagging chromosomes. In merotelic attachments, MCAK activity reinforces the effect microtubules have on the kinetochore. Parts of the kinetochore that are pulled away from Aurora B, remain unphosphorylated leading to activity of MCAK, which in turn strengthens the pulling forces. The parts of the kinetochore that are phosphorylated by Aurora B contain inactive MCAK and the attachments on these parts are lost upon KMN phosphorylation.

In conclusion, merotelic and syntelic attachments have to be corrected to achieve proper bi-orientation. Although these attachments are dependent on similar mechanisms for correction, the exact processes differ slightly and makes them fascinating to study.

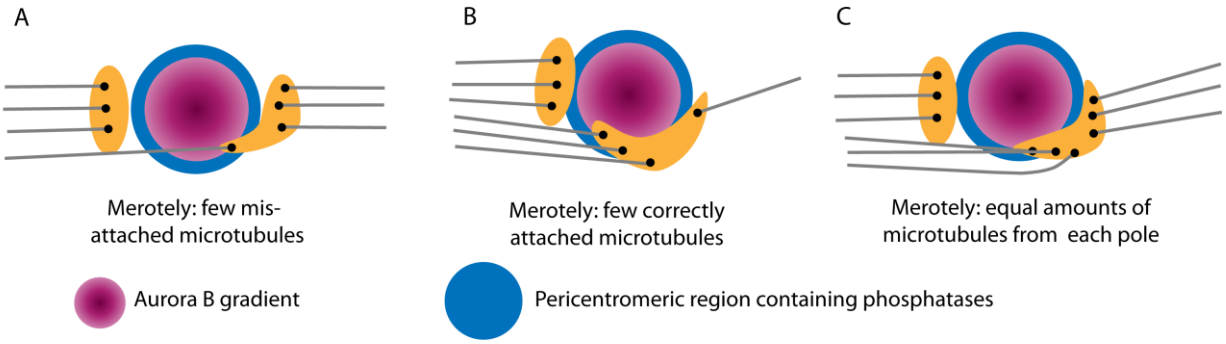

Figure 3. The ratio of correctly bound microtubules versus incorrectly bound microtubules at merotelic kinetochores determines how these kinetochores are corrected. The more microtubules from the incorrect pole, the more the kinetochore is pulled towards the Aurora B gradient and pericentromeric regions containing protein phosphatases that activate MCAK, the more the chance that kinetochore-microtubule interactions are lost. However, because of the flexible nature of the kinetochore, it could be so that only parts of the kinetochore reach the Aurora B gradient and the pericentromeric regions.

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