

The Kinetochore and Mitosis: Focus on the Regulation and Correction Mechanisms of Chromosome-to-Microtubule Attachments

Rita M. Reis¹ and Hassan Bousbaa^{1,2}

*¹Centro de Investigação em Ciências da Saúde (CICS),
Instituto Superior de Ciências da Saúde Norte/CESPU,*

*²Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP),
Portugal*

1. Introduction

During mitosis, accurate segregation of the chromosomes duplicated at the S phase of the cell cycle relies on the successful of their proper attachment to microtubules of the mitotic spindle and their alignment at the metaphase plate before the onset of anaphase. Attachment errors or failure to align can lead to chromosome gains or losses, a condition known as chromosome instability (CIN) which is a common feature amongst many cancers.

The kinetochore, a multiprotein structure assembled on the centromeres, plays a key role in chromosome attachment and high fidelity of mitotic chromosome segregation between daughter cells.

The kinetochore structure provides the attachment site of microtubule polymers to chromosome. Each mitotic chromosome has a pair of kinetochores, positioned on opposite sides of the centromere, that allow for its bipolar attachment to the spindle. Besides this role in attachment, the kinetochore controls the metaphase-anaphase transition by inhibiting chromatid separation until all chromosomes are properly attached and aligned at the metaphase plate, thereby ensuring equitable sharing of chromosomes upon cell division.

During the last decade, considerable progress has been made in understanding the molecular composition of the kinetochore, shedding light on the higher order organization of the kinetochore and on its activity in mediating chromosome attachment to spindle microtubules and in regulating the fidelity of the metaphase to anaphase transition. We address these aspects, concentrating in particular on the role that the kinetochores have in sensing and resolving aberrant kinetochore-microtubule attachments and in preventing chromosome missegregation.

2. The events of Mitosis

In 1879, Walther Flemming was the first to describe in great detail cell division. The Fleming's great discovery was that during the cell division one longitudinal half of each

chromosome goes in each direction, so that each daughter nucleus is formed from a complete set of longitudinal halves. In 1882 he termed this process Mitosis (from the Greek *mitos* “thread-metamorphosis”) (Baker, 1949; Paweletz, 2001).

2.1 Mitotic phases

Nowadays, it is known that Mitosis is a complex and highly regulated process, used by eukaryotes to generate two identical daughter cells from one original mother cell. This particular phase of the cell cycle, ensures that the cell faithfully segregates the sister chromatids, of the duplicated chromosomes, into the daughter cells, producing two cells that are identical to one another and to the original parent cell. Conventionally, mitosis is divided into five stages: Prophase, Prometaphase, Metaphase, Anaphase and Telophase (Fig. 1).

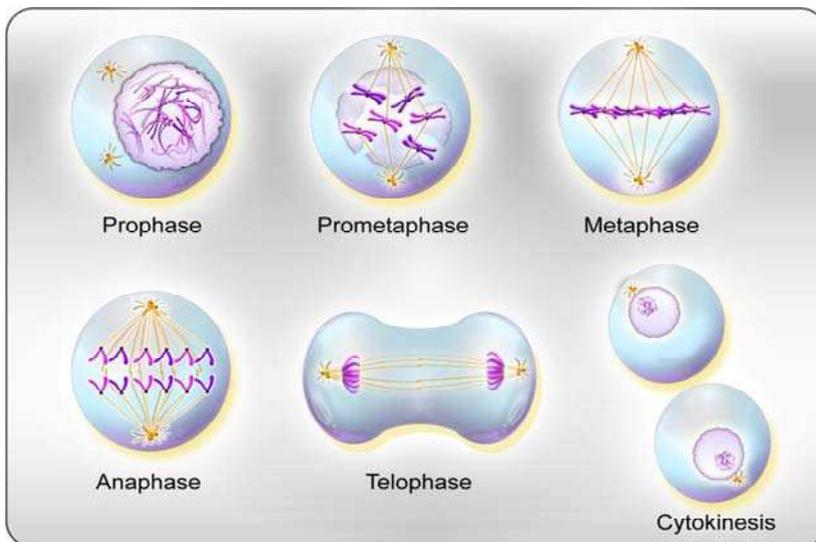


Fig. 1. Mitotic stages and Cytokinesis of animal cells. Prophase: chromatin condenses into chromosomes and centrosomes start to separate; Prometaphase: begins with nuclear envelope breakdown, chromosomes are captured by microtubules that are growing from opposite poles of the spindle; Metaphase: all chromosomes align at the equator of the cell; Anaphase: sister chromatids move to opposite poles; Telophase: sister chromatids reach the poles, decondense and nuclear membrane reforms around each identical daughter nuclei. Simultaneously, the division of the cytoplasm generates two independent daughter cells by a process called Cytokinesis.

Prophase, is marked by the appearance of condensed chromosomes that emerge as two identical filaments (sister chromatids, produced in the S phase of the cell cycle) and the centrosomes begin to separate to opposite sites initiating the mitotic spindle assembly. The next stage, Prometaphase, starts with the nuclear envelope breakdown allowing that microtubules, from the opposite centrosomes, occupy all the cytoplasmic space and fully

organize the mitotic spindle. These microtubules bind the chromosomes and help them to reach and align at the equatorial cell region; at this stage the cell has achieved the Metaphase. In Metaphase, chromosomes are aligned at the middle of the spindle forming the metaphase plate, awaiting the signal to the next stage, Anaphase. This transition, from Metaphase to Anaphase, is the most critical event of the cell cycle. This event occurs when the cohesion between sister chromatids is dissolved and the separated sisters migrate to opposite poles of the spindle. The last stage of mitosis is Telophase, during which the spindle disassembles, the chromosomes decondense and the nuclear envelop reappears. Simultaneously, the division of the cytoplasm generates two independent interphase daughter cells by a process called Cytokinesis. If these events do not occur properly and in the right sequence, the newly formed cells either die or carry on genetic damages that can lead ultimately to cancer.

2.2 Regulation of Mitosis

The timing and coordination of Mitosis progression relies mostly on mechanisms of protein Phosphorylation and Proteolysis (Nigg, 2001). While Phosphorylation is a reversible protein modification, and thus ideal for the control of reversible mitotic processes such as spindle assembly, Proteolysis, in contrast, by its chemical irreversibility, is more appropriate for controlling events that must not be reversed such as sister-chromatid separation (King et al., 1996; Morgan, 2007). The Mitosis-Promoting Factor (MPF), first called Maturation-Promoting Factor, was described as the “entity” whose activity controls entry into Mitosis (Masui & Markert, 1971). Now, it is well-established that the MPF is a heterodimer composed by one molecule of cyclin B and one of Cdc2 (cell division cycle). Later, Cdc2 became known as Cdk1 since it was established, at the Cold Spring Harbor Symposium on the Cell Cycle in 1991, that kinases that are associated with cyclins should be called “cyclin dependent kinases” or Cdks (Doree & Hunt, 2002). Cyclin B-Cdk1 (MPF) accumulates before entry into mitosis and its activation leads to phosphorylation of several substrates responsible for the morphological changes that occur in early stages of mitosis such as nuclear envelop breakdown, centrosome separation, spindle assembly, chromosome condensation, and endoplasmic reticulum and Golgi fragmentation. However, proteolysis-mediated disassembly of Cyclin-Cdk1 complexes is required for mitotic exit and cytokinesis (Nigg, 2001). Besides the direct role in regulating Cdk activity by controlling cyclin levels, proteolysis also drives cell cycle progression by directly triggering some key cell cycle events such as sister chromatids separation at metaphase-anaphase transition, thus providing directionality of the cell cycle (King et al., 1996). Therefore, these two mechanisms, phosphorylation and proteolysis, are interdependent since proteolysis events are controlled by phosphorylation and the mitotic kinases (M-Cdks) are inactivated by proteolytic destruction of cyclins (King et al., 1996; Morgan, 2007; Nigg, 2001).

The major events of mitosis are sister-chromatid separation and segregation. If these processes do not occur accurately the result would be the production of cells with extra or missing chromosomes, a state known as aneuploidy, which is a common characteristic of cancer cells (Holland & Cleveland, 2009; Schwartzman et al., 2010). To avoid the occurrence of aneuploidy, cells have developed a control system called mitotic checkpoint

or Spindle Assembly Checkpoint (SAC), which prevents the cell entry in anaphase until all chromosomes are correctly aligned, forming the metaphase plate, with proper attachment to the mitotic spindle (Rieder et al., 1995) and under a certain tension (Nicklas et al., 1995). When these conditions are satisfied, SAC is turned off. This checkpoint arrests cells in mitosis by blocking protein degradation. With its inactivation, the Anaphase Promoting Complex or Cyclosome (APC/C), an ubiquitin protein ligase whose activity depends on the activator protein Cdc20, targets Securin and Cyclin B for ubiquitylation and posterior proteolysis through the 26S proteasome. Destruction of Securin turns on Separase which cleaves the cohesion complex that holds sister chromatids together; destruction of Cyclin B leads to anaphase onset (Zachariae & Nasmyth, 1999).

2.3 The mitotic spindle

In order to congress and align at the center of the cell, and then segregate its sister chromatids to opposite poles, chromosomes use the mitotic spindle. The mitotic spindle is organized in a symmetric and fusiform structure composed of microtubules, polymers made of α - and β -tubulin heterodimers that being all oriented in the same way create a polar nature with β -tubulin exposed at one end (plus-end) and α -tubulin at the other end (minus-end) (Desai & Mitchison, 1997). Depending on the position of the microtubule plus-ends, spindle microtubules can be divided into three classes: astral-microtubules, interpolar-microtubules and kinetochore-microtubules; all contribute to the bipolarity of the mitotic spindle. Astral microtubules, emanate from the spindle poles and radiate out throughout the cytoplasm with the plus-ends interacting with the cell cortex. Interpolar microtubules, extend from the spindle poles to the spindle midzone where their plus-ends form an interdigitating system that connects the two spindle poles. Kinetochore microtubules, connect the spindle poles to chromosomes with the minus ends near the poles and the plus ends binding laterally or end-on, specifically to the kinetochores (an intricate protein complex raised on the centromeric DNA) (Hayden et al., 1990; Merdes & De Mey, 1990; Rieder & Alexander, 1990). These kinetochore-microtubules form a morphologically distinct bundle denominated K-fiber (kinetochore-fiber), made of up to 30 kinetochore-attached microtubules in higher eukaryotes, which is directly involved in chromosome congression and sister-chromatid segregation (Rieder & Salmon, 1998).

The ability of spindle microtubules to quickly assemble and disassemble (dynamic instability) provides them the necessary behavior to capture chromosomes. This statement becomes the basis of the first and favorite model for spindle assembly in systems with centrosomes, and is known as "search and capture" (Kirschner & Mitchison, 1986; Mitchison & Kirschner, 1984). This model postulates that, when nuclear envelop breaks down, chromosomes become accessible to microtubules radiated from centrosomes, which, through their dynamic nature, randomly explore the cytoplasm until capture a kinetochore, laterally or with the plus-end, forming an attachment that stabilizes the microtubule (Mitchison et al., 1986; Nicklas & Kubai, 1985). Although the plus-ends of microtubules can bind directly the kinetochore, the first contact usually occurs laterally. After binding one of the unattached sister kinetochores, the chromosome is rapidly transported along the side of the microtubule towards the spindle pole, in a mechanism

that is independent of microtubule depolymerization and involves the minus-end directed motor protein Dynein (Rieder & Alexander, 1990; Yang et al., 2007). Since the spindle pole has a high microtubule density, additional microtubules from the same pole will attach to the kinetochore, and the plus-ends of laterally associated microtubules shorten until reach the end-on binding, resulting in a stable K-fiber (Rieder, 2005). Since this model is based on "search and capture" of astral microtubules that radiate from centrosomes, it is not valid to cells lacking centrosomes, like higher plants and many animal oocytes. An alternative model, called "spindle self organization", proposes that microtubules are nucleated in the vicinity of chromatin, elongate and then, helped by motor proteins, are sorted into a bipolar array and focused at the poles (Walczak et al., 1998). Indeed, it was shown that cells with centrosomes, besides the "search and capture" mechanism, also use this chromosome-driven K-fiber formation for spindle assembly (Khodjakov et al., 2000; Maiato et al., 2004). In these "combined" system, the K-fibers nucleated from chromosomes are integrated at the spindle assisted by astral microtubules that search, capture and transport them toward the pole in a dynein-dependent manner (Khodjakov et al., 2003; Maiato et al., 2004). Several studies, in *Xenopus* extracts and in mammalian cells, show that the chromosome-driven microtubule formation relies in a Ran-GTP concentration gradient around mitotic chromosomes, which in turn induces a gradient of proteins that regulate microtubule nucleation/dynamics, by dissociating them from importin- β (Fuller, 2010; Kalab et al., 2002; Tulu et al., 2006). Furthermore, studies in mammalian somatic cells support a model in which the kinetochores are the key players in this chromosome-mediated spindle assembly (O'Connell et al., 2009). Another mechanism that contributes to spindle formation is the "search and transport", in which peripheral microtubules, similarly to K-fibers nucleated from chromosomes, are transported through aster microtubules to the poles, where they are incorporated into the spindle (O'Connell & Khodjakov, 2007; Tulu et al., 2003).

For a successful cell division, chromosomes must interact with spindle microtubules. Through their dynamic behavior, microtubules allow that chromosomes congress to the equatorial region of the spindle forming the metaphase plate and, are responsible for the segregation of sister chromatids to opposite poles. There are three major forces acting on chromosomes that drivethese movements: (1) The "Polar ejection force" or "Polar wind" that is generated by non-kinetochore microtubules and pushes chromosomes away from the spindle poles (anti-poleward movement) (Kapoor & Compton, 2002; Rieder et al., 1986); (2) "Microtubule flux", which consists in a flow of tubulin subunits from the spindle equator to the spindle poles as consequence of polymerization at the plus-ends, depolymerization at minus-ends, and translocation of the entire microtubule toward the spindle pole (Cassimeris, 2004; Mitchison, 1989); and (3) "Pacman" mechanism, in which the kinetochores catalyze the depolymerization of kinetochore-microtubule plus-ends resulting in chromosomal movement to the spindle poles by "chewing up" the microtubule track (Gorbsky et al., 1987; Mitchison et al., 1986).

3. The kinetochore structure

In 1894, Metzner was the first investigator to describe the "kinetic region", a specific chromatin area, located at the primary constriction on each side of the chromosome, that

leads the way of sister chromatids during the poleward motion (Metzner, 1894; Rieder, 2005; Schrader, 1944). Later, in 1934, Sharp coined these structures as "kinetochores", from the Greek 'kineto-' meaning 'move' and '-chore' meaning 'means for distribution' (Rieder, 2005; Schrader, 1936). In 1960, Bill Brinkley was the first to describe the mammalian kinetochore structure as a trilaminar proteinaceous disc structure that flanked the centromere: an electron-dense inner plate located on the surface of the centromeric heterochromatin, separated from an electron dense outer plate by a lighter middle layer (Brinkley & Stubblefield, 1966). However, using high-pressure frozen specimens, this electron-translucent middle layer is not visible, suggesting that it is an artifact produced during the classical EM fixation and/or dehydration procedures (McEwen et al., 1998). In 1967, Jokelainen shows the existence of a corona of electron opaque substance that covers the outer kinetochore layer, which after microtubule binding, becomes hard to detect by EM (Cassimeris et al., 1990; Jokelainen, 1967)(Fig. 2).

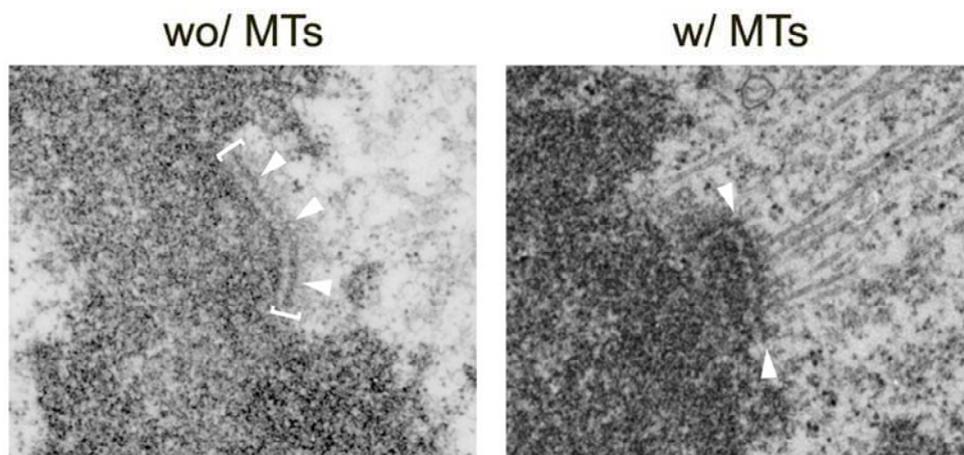


Fig. 2. Electron micrographs of kinetochores, from PTK1 cells, in absence and presence of microtubules. The trilaminar structure of kinetochore (brackets) is well defined without microtubules (wo/ MTs) and becomes distorted with microtubules (w/ MTs) embedded within the outer kinetochore plate (arrowheads). Courtesy of Dr. Helder Maiato (Maiato et al., 2006).

These mature kinetochores, with the trilaminar structure, occurs only in prometaphase after nuclear envelop breakdown. The kinetochore is built on the centromeric region of each sister chromatid by the assembly of multiprotein complexes (Fig. 3). In early G1 the typical layer conformation disappears giving rise to a condensed structure that unfolds in late G1 forming a linear, bead-like conformation that persists until S-phase, where it transforms into a loose fibrous bundle that duplicates at late S-phase. In late G2, pre-kinetochores refold into two separated and condensed structures. During prophase, these duplicated pre-kinetochores differentiate at the primary constriction of the sister chromatids originating the kinetochore layers at the time of nuclear envelop breakdown, completing the cycle (He & Brinkley, 1996).

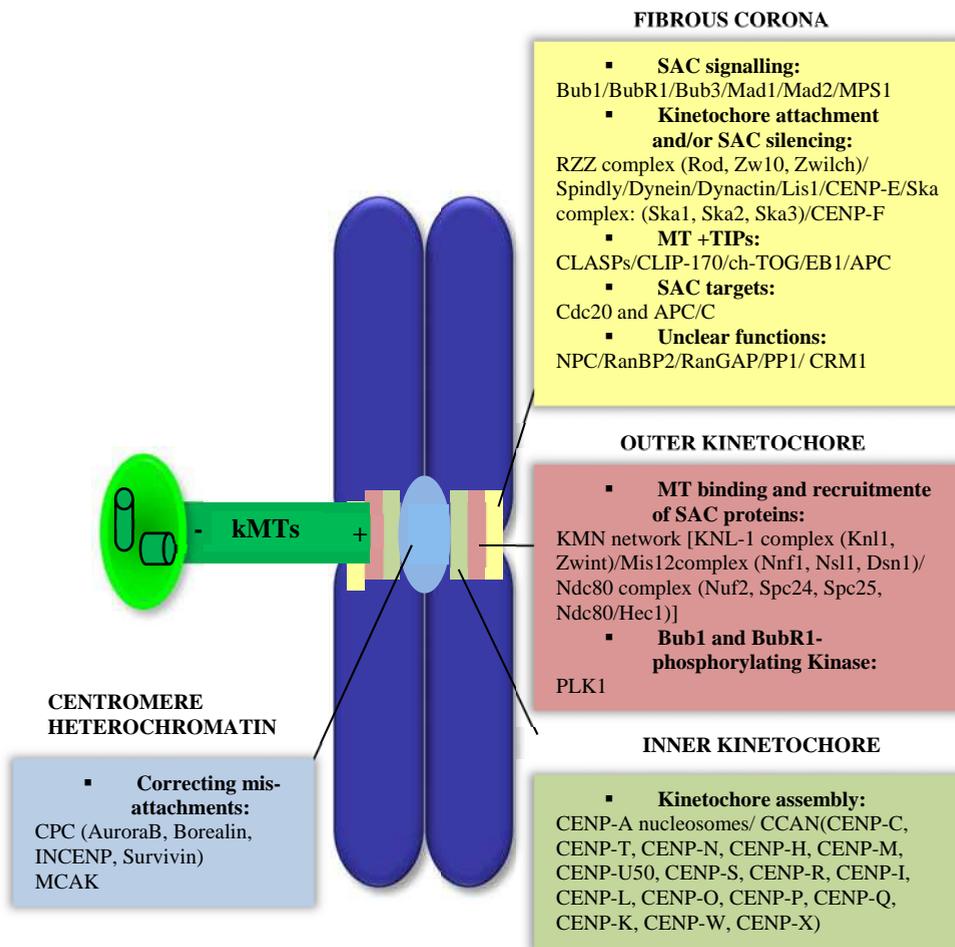


Fig. 3. Overview of protein complexes that build the kinetochore in animal cells. The kinetochore is built on the centromere as a trilaminar protein-rich structure: the inner kinetochore, the outer kinetochore and the fibrous corona. The inner and outer kinetochore layers are separated by the translucent interzone (not depicted). Proteins at centromere heterochromatin and at each kinetochore layer are indicated; the list is not exhaustive as it is continuously growing. Adapted from (Silva et al., 2011).

Kinetochores localize at the constriction region on each sister chromatid, assembling on the centromere, from the Greek ‘centro-’ (meaning ‘central’) and ‘-mere’ (meaning ‘part’), a specific chromatin region with distinct epigenetic marks (Cheeseman & Desai, 2008). In this particular region, the nucleosomal histone H3 is replaced by the variant CENP-A (CENTromere Protein A)(Vafa & Sullivan, 1997). Although it seems consensual that CENP-A is essential for specifying the site for kinetochore formation, the role of CENP-A in kinetochore assembly is still unclear (Bowers & Mellone, 2011). Some studied, using

overexpression/mistargeting of CENP-A show that CENP-A is not sufficient to originate functional kinetochores (Gascoigne et al., 2011; Van Hooser et al., 2001). Other studies, using ectopic targeting of HJURP (a CENP-A chromatin assembly factor) (Barnhart et al., 2011) and using *in vitro* kinetochores (Guse et al., 2011), demonstrate that CENP-A is indeed sufficient to form functional kinetochores. Besides CENP-A, several other proteins are present at vertebrate centromeres throughout the cell cycle. They were named CCAN for Constitutive Centromere Associated Network (Cheeseman & Desai, 2008). CCAN comprises at least 16 proteins, CENP-C, CENP-H, CENP-I, CENP-K-U, CENP-W, and CENP-X, grouped into several subcomplexes (Amano et al., 2009; Santaguida & Musacchio, 2009). It is known that whereas CENP-C, CENP-N and CENP-K associate specifically with CENP-A nucleosomes (Carroll et al., 2009; Guse et al., 2011), CENP-T/W complex are DNA-binding proteins that associate with histone H3 nucleosomes in the centromeric region (Guse et al., 2011; Hori et al., 2008). Although only few proteins are present at centromeres throughout the cell cycle, during mitosis this number increases substantially, indicating the existence of a complex assembly regulatory process. Gascoigne et al. have contributed with a piece of this puzzle, by demonstrating that phosphorylation of CENP-T by CDK can control kinetochore assembly in vertebrates. They saw that CENP-T becomes phosphorylated in G2, has a maximum at metaphase and drops until anaphase, and show that preventing CENP-T phosphorylation abolishes the recruitment of Ndc80 (Hec1 in mammals) (Gascoigne et al., 2011). Ndc80 is part of the Ndc80 complex that in turns is part of the so called KMN network, the microtubule-binding core of kinetochore, composed by the two-subunit Knl1 complex (containing Knl1/Blinkin and Zwint), the four-subunit complex Mis12 (containing Mis12, Dsn1, Nnf1 and Nsl1) and the four-subunit Ndc80 complex (containing Ndc80, Nuf2, Spc24 and Spc25). In the Ndc80 complex, Nuf2 and Ndc80 localize to the outer kinetochore region and bind directly to microtubules, and Spc24 and Spc25 localize at the inner kinetochore region and bind to the Mis12 and Knl1 complexes (Santaguida & Musacchio, 2009). A recent study, demonstrate that a conserved motif in the N-terminal region of Cenp-C binds directly to the Mis12 complex (Screpanti et al., 2011). In fact, several proteins from the CCAN, such as CENP-C, CENP-H, CENP-K, CENP-I, CENP-T/W, and CENP-X have been implicated in the recruitment of KMN proteins (Petrovic et al., 2010). These connections between the CCAN network and the KMN network link the inner to the outer kinetochore. KMN complex was considered as the essential core of the kinetochore. Besides the direct binding of centromeres to microtubules, it interacts, directly or indirectly, with proteins that are involved in crucial mitotic functions such as, regulation of the activity of spindle assembly checkpoint and kinetochore-microtubule interactions (Cheeseman & Desai, 2008; Przewloka & Glover, 2009). It allows, directly or indirectly, the recruitment/interaction of regulatory proteins such as Microtubule associated proteins (like CLASP, CLIP170, EB1, APC), motor proteins (like CENP-E, dynein/dynactin complex), protein involved in spindle assembly checkpoint (like Mad1, Mad2, Bub1, Bub3, BubR1, Bub1, RZZ complex, Mps1). It also interacts with the SKA (Spindle and Kinetochore Associated) complex, thought to be involved in stable end-on kinetochore-microtubule attachment; and with the CPC (Chromosome Passenger Complex- Aurora B, INCENP, Borealin and Survivin) complex, among others (Fig. 3). Indeed, depletion of any protein from the KMN network, in all eukaryotes, result in abnormal kinetochore structure or in a lack of kinetochore-microtubule attachment (Lampert & Westermann, 2011; Przewloka & Glover, 2009; Santaguida & Musacchio, 2009).

4. Kinetochore functions

The Kinetochores are dynamic structures with more than 100 proteins organized into networks of several complexes of probably intertwined functions during cell division (Cheeseman & Desai, 2008). They attach sister-chromatids to microtubules of the bipolar mitotic spindle and position the chromosome at the spindle equator; they inhibit anaphase onset until all chromosomes are properly attached and aligned at the metaphase plate; and provide correction mechanism of erroneous attachments. Next, we address the overall mechanisms and the molecular link between chromosome attachment to spindle microtubules, correction of attachment errors, and the spindle assembly checkpoint.

4.1 Kinetochores mediate bipolar attachment to the spindle

High-fidelity chromosome segregation at the onset of anaphase relies on the success of sister kinetochore bi-orientation on the mitotic spindle (Tanaka, 2010). Chromosome bi-orientation is a step-wise process that sequentially involves initial interaction of a kinetochore with the lateral surface of a microtubule; transport of the kinetochore to a spindle pole; conversion from the lateral to the end-on attachment; and attachment of the sister kinetochore to microtubules from the opposite spindle pole (Fig. 4).

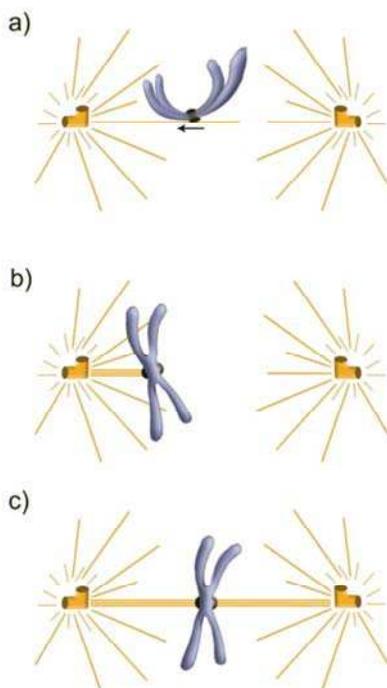


Fig. 4. Steps towards chromosome bi-orientation. Upon nuclear envelope breakdown, chromosome bi-orientation sequentially involves a) initial interaction of a kinetochore with the lateral surface of a microtubule and transport of the kinetochore to a spindle pole; b) conversion from the lateral to the end-on attachment; and c) attachment of the sister kinetochore to microtubules from the opposite spindle pole to achieve bipolar attachment.

As stated above, initial encounter of spindle pole microtubules with the kinetochore is mediated by the so-called “search and capture” process, guided by a Ran-GTP concentration gradient around the chromosome (Clarke & Zhang, 2008; Wollman et al., 2005). Additionally, and to avoid further delay in this initial encounter, kinetochore can nucleate microtubules which, by interacting with spindle-pole nucleated microtubules along their length, facilitate kinetochore loading onto centrosome-nucleated microtubules (Kitamura et al., 2010). The first step of the “search and capture” process is the interaction of the kinetochore with the lateral surface of a microtubule, called the lattice, followed by chromosome sliding along the microtubule lattice towards a spindle pole. The poleward kinetochore transport, powered by the minus end-directed dynein motor protein complex, brings chromosomes scattered throughout the cytoplasm to the mitotic spindle area (Sharp et al., 2000; Yang et al., 2007). Dynein binding to kinetochores requires the protein Spindly which in turn requires the RZZ complex [made of the proteins Rough-deal (ROD), Zeste-white (ZW10), and Zwilch] to localize to kinetochore (Chan et al., 2009; Griffis et al., 2007; Karess, 2005). The connection to the essential microtubule-binding core of the kinetochore is mediated by the protein Zwint-1 that links the RZZ complex to the KMN network (Wang et al., 2004).

During the association of the kinetochore with the microtubule lattice, shrinking of the microtubule plus-end leads to kinetochore tethering at the microtubule plus-end (end-on attachment) and to its further transport towards the spindle pole. End-on attachments are more robust than lateral attachments and are critical for bi-orientation and for the generation of load-bearing attachments (Joglekar et al., 2010). The mechanism of the conversion from a lateral into an end-on attachment remains unclear. Proteins thought to be required for this conversion include the *C. elegans* RZZ complex and Spindly/SPDL-1 (Gassmann et al., 2008), the vertebrate Ska1-3 (Gaitanos et al., 2009; Guimaraes & Deluca, 2009), and the *Saccharomyces cerevisiae* Ndc80 loop region (Tanaka, 2010).

The chromosome reaches the spindle pole with one kinetochore end-on attached to k-fiber microtubules from that pole and its sister kinetochore unattached, a state known as monotelic attachment. Aided by the back-to-back kinetochore geometry, the unattached kinetochore becomes attached when captured by microtubule searching from the opposite pole, thereby leading to chromosome bi-orientation (amphitelic attachment) (Fig. 4). Subsequently, k-fiber microtubule shrinking and elongation promote congression of the chromosomes towards the spindle equator in order to form the metaphase plate (Silva et al., 2011). As an additional mechanism that leads to bi-orientation, chromosomes can be transported towards the spindle equator by gliding alongside microtubules attached to other already bi-oriented chromosomes, driven by kinetochore-bound CENP-E, a plus end-directed microtubule motor of the kinesin-7 family (Kapoor et al., 2006).

4.2 Correcting aberrant kinetochore-microtubule attachments

Although the back-to-back orientation of sister kinetochores imposes a geometric constraint that favors chromosome bi-orientation, errors in kinetochore-microtubule attachments are frequent due to the stochastic nature of the search and capture mechanism. Such errors include monotelic (one kinetochore unattached while its sister attached to one spindle pole), syntelic (two sister kinetochores bound to microtubules from the same pole), and merotelic (one sister kinetochore bound to microtubules from both poles) attachments (Fig. 5) (Silva et al., 2011). Most of these errors occur at the beginning of prometaphase and, if left uncorrected, would lead to unequal chromosome segregation and aneuploidy (Kops et al., 2005).

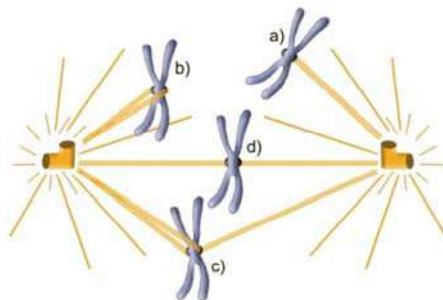


Fig. 5. Possible attachment errors during chromosome bi-orientation event. Errors include a) monotelic, with one kinetochore unattached while its sister attached to one spindle pole; b) syntelic, with two sister kinetochores bound to microtubules from the same pole; and c) merotelic, with one sister kinetochore bound to microtubules from both poles attachments. These errors are generally converted to d) amphitelic attachments, the only correct attachment configuration, by the error correction machinery.

Error correction is the result of biochemical changes induced by mechanical forces acting on the kinetochore. Bi-oriented kinetochores (amphitelic attachment) are under tension that results from the pulling forces of spindle microtubules in opposite directions. Cohesion between sister centromeres is necessary to generate this tension (Tanaka et al., 1999). Tension across the centromere stabilizes kinetochore-microtubule attachments, as evidenced by Bruce Nicklas in his classical micromanipulation experiments with insect spermatocytes (Nicklas & Koch, 1969; Nicklas & Ward, 1994). Tension artificially applied with a glass microneedle on grasshopper spermatocyte chromosomes stabilizes unipolar attachments by increasing the occupancy of microtubule attachment sites.

The first evidence of the translation of mechanical forces (tension) acting on the kinetochore into biochemical changes was provided by the identification of the Ipl-1 kinase in the budding yeast *Saccharomyces cerevisiae* (Biggins et al., 1999). In Ipl-1 defective yeast cells, kinetochores can interact with microtubules but sister-kinetochores often fail to bi-orient, suggesting that Ipl-1 promotes bi-orientation. Interestingly, in the same mutant, kinetochore-microtubule attachments are stabilized in the absence of tension, suggesting that Ipl-1 promotes bi-orientation by destabilizing tensionless attachments (Dewar et al., 2004; Tanaka et al., 2002). Defective Aurora B kinase (the mammalian functional homolog of Ipl-1) induces an increase in syntelic attachments, suggesting a similar work in mammalian cells (Hauf et al., 2003; Kallio et al., 2002). Aurora B localizes to the inner centromere, as the catalytic component of the chromosome passenger complex (CPC), together with the binding partners INCENP, Survivin, and Borealin (Ruchaud et al., 2007). While the molecular mechanism of error detection and correction is not fully understood, a current model proposes that Aurora B kinase promotes the turnover of erroneous kinetochore-microtubule attachments through phosphorylation of its substrates at the outer kinetochore, in a tension-dependent manner (Lampson & Cheeseman, 2011). In this so-called spatial model, the distance between Aurora B and its outer kinetochore substrates is a critical determinant for their phosphorylation. When tension is low or absent, outer kinetochore substrates are phosphorylated due to their proximity to an Aurora B kinase activity gradient around the inner centromere (Wang et al., 2011), which destabilizes kinetochore-

microtubule attachments. For instance, in higher eukaryotes, Aurora B phosphorylation of the Ndc80 complex was reported to weaken its affinity to microtubules, while phosphorylation of the microtubule depolymerase kinesin MCAK activates its depolymerase activity (Cheeseman et al., 2006; Lan et al., 2004). Both changes promote destabilization of tensionless kinetochore-microtubule attachments, providing a further opportunity for chromosome to bi-orient. Bi-orientation locates outer kinetochore substrates away from the Aurora B activity gradient and close to the opposing and constitutively active phosphatase. In this way, dephosphorylation of Ndc80 and MCAK stabilizes amphitelic kinetochore-microtubule attachments. Therefore, tension provides the determinant for the error correction machinery to distinguish syntelic and merotelic from amphitelic attachments. Once all chromosomes become bi-oriented and aligned at the metaphase plate, Aurora B leaves the inner centromere and become concentrated on the spindle midzone. This presumably prevents turnover of kinetochore-microtubules attachments during anaphase, in which tension is reduced.

4.3 The spindle assembly checkpoint

Cells must be maintained in mitosis, by preventing securin and cyclin B degradation, until all chromosomes are properly bi-oriented and aligned at the metaphase plate. This is achieved by the evolutionary conserved mechanism called The "Spindle Assembly Checkpoint" (SAC) that prevents the E3 ubiquitin ligase APC/C (Anaphase Promoting Complex/Cyclosome) from targeting securin and cyclin B for degradation by the 26S proteasome, as long as unattached or improperly attached chromosomes are present (Silva et al., 2011).

Components of the SAC are conserved from yeast to human and include the core SAC proteins Mad1, Mad2, Bub1, Bub3, Mad3/BubR1, and the protein kinases Mps1 and Aurora B (Musacchio & Salmon, 2007). It is widely accepted that the SAC inhibits the APC/C through the Mad2 protein that sequesters Cdc20 an essential activator of the APC/C individually or in an inhibitory complex with BubR1 and Bub3, forming the "Mitotic Checkpoint Complex" (MCC) (Kallio et al., 2002; Sudakin et al., 2001). Unattached kinetochore is believed to provide the platform for the generation of this diffusible "wait anaphase" signal that inhibits mitosis. Indeed, checkpoint proteins dynamically associate with unattached kinetochores, reflecting the catalytic assembly and then release and diffusion of MCC. However this model is not universal as MCC formation does not require a kinetochore in yeast (Fraschini et al., 2001).

SAC becomes satisfied once all chromosomes are correctly bi-oriented at the metaphase plate. In order for cell to proceed to anaphase, the production of MCC must be halted and existing MCC must be disassembled. This is referred to as "SAC silencing", thought to be mediated by several mechanisms at least in mammalian cells (Fuller & Stukenberg, 2009; Vanoosthuyse & Hardwick, 2009). Kinetochore-mediated production of MCC is suggested to be stopped by Dynein-driven transport of SAC proteins from attached kinetochores towards spindle poles, along microtubules (Howell et al., 2001); and through dissociation of Mad2/Cdc20 complex due to competition binding of the protein p31^{comet} to the dimerization interface of Mad2. Disassembly of existing MCC is promoted by p31^{comet}- and UbcH10-dependent Cdc20 autoubiquitination (Reddy et al., 2007; Stegmeier et al., 2007), and proteasomal degradation (Ma & Poon, 2011). The coordination between these mechanisms

during SAC inactivation and mitotic exit remains unclear. Once SAC is turned off, APC/C becomes activated and targets securin and cyclin B for degradation by the proteasome, thereby promoting sister-chromatid separation and exit from mitosis, respectively.

4.4 Relationship between microtubule attachment, attachment error correction, and SAC

The role of SAC in delaying anaphase onset, in the presence of unattached or improperly attached kinetochores, foresees the existence of a dynamic relationship between microtubule attachment, error correction machinery, and checkpoint signaling. This relationship could efficiently assure that attachment errors are detected; checkpoint signals are produced; and attachment errors are corrected only at the kinetochore platform, being all these activities in the right place at the right time.

A molecular link between microtubule attachment machinery and SAC activity has been suggested by the phenotype of yeasts with defective Ndc80 (Ndc80, Nuf2, Spc24, and Spc25) complex, a component of the KMN supercomplex (the core microtubule-binding interface of kinetochores). These mutants fail to attach chromosomes to the spindle and to activate SAC (Burke & Stukenberg, 2008; McClelland et al., 2003). In metazoans, the relationship between kinetochore-microtubule binding and SAC signaling is further suggested by the observation that KMN, through the Ndc80 complex, is required for kinetochore assembly of SAC proteins as well as for the generation of a SAC signal (Burke & Stukenberg, 2008). In addition, Blinkin (the human homologue of yeast kinetochore protein Spc105) directs Bub1 and BubR1 to kinetochores through interaction with their TPR domains (Bolanos-Garcia et al., 2011; Kiyomitsu et al., 2007). Preventing interaction between the Bubs and Blinkin, either by siRNA or by point mutation of the TPR domain of Bubs, abolishes the generation of SAC signals, suggesting that Blinkin has a role both in microtubule attachment and SAC signaling (Kiyomitsu et al., 2007). The SAC proteins Bub1, BubR1, Bub3, and Mps1 were themselves involved in the regulation of microtubule attachment, beside their role in SAC signaling (Logarinho & Bousbaa, 2008).

The highly conserved serine/threonine Aurora B kinase provides the main link between the error correction machinery and SAC. By destabilizing tensionless kinetochore-microtubule attachments, Aurora B creates unattached kinetochores that can be filled with checkpoint proteins to generate SAC signals (Burke & Stukenberg, 2008). This in turn allows time for error correction and bi-orientation. The direct involvement of Aurora B activity in SAC control is suggested by its requirement for SAC protein recruitment to unattached kinetochores (tensionless) artificially created in cells treated with the microtubule-depolymerizing drug nocodazole (Kallio et al., 2002). Moreover, recent studies strongly suggest that Aurora B directly contributes to SAC signaling independently of its error correction activity (Santaguida et al., 2011).

5. Conclusion

Accurate chromosome segregation during mitosis relies on the activities of the kinetochore. Here, we highlighted the event of mitosis and focused on the structure and functions of kinetochore in chromosome attachment, chromosome movement, error correction, and the generation of inhibitory signals that prevent anaphase in the presence of attachment errors.

The basic mechanisms of these kinetochore functions, their interplays, and regulatory pathways remain under investigation. Elucidating these mechanisms is crucial for future progress and is relevant to cancer aetiology and therapy. Indeed, failure in any of these kinetochore functions can lead to chromosome missegregation, with chromosome losses and gains, which may contribute to the aneuploidy phenotype that characterizes many cancers (Kops et al., 2005; Thompson et al., 2010).

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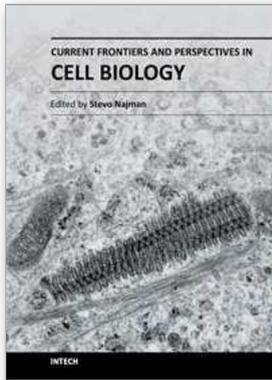
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Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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