

Bi-orienting chromosomes: acrobatics on the mitotic spindle

Tomoyuki U. Tanaka

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Abstract To maintain their genetic integrity, eukaryotic cells must segregate their chromosomes properly to opposite poles during mitosis. This process mainly depends on the forces generated by microtubules that attach to kinetochores. During prometaphase, kinetochores initially interact with a single microtubule that extends from a spindle pole and then move towards a spindle pole. Subsequently, microtubules that extend from the other spindle pole also interact with kinetochores and, eventually, each sister kinetochore attaches to microtubules that extend from opposite poles (sister kinetochore bi-orientation). If sister kinetochores interact with microtubules in wrong orientation, this must be corrected before the onset of anaphase. Here, I discuss the processes leading to bi-orientation and the mechanisms ensuring this pivotal state that is required for proper chromosome segregation.

Introduction

In trapeze acrobatics, performers use two wired swings, each hung from ceiling, and skillfully jump from one swing to the other with perfect timing. Such acrobatics require elaborate training and, if performed splendidly, win much applause. However, similar acrobatics are secretly performed by chromosomes within our cells, without acknowledgement or applause. Chromosomes use microtubules, instead of wired swings, and often change their associated micro-

tubules until proper association is established. During this process, the main microtubule attachment sites on chromosomes are provided by kinetochores, large protein complexes formed at centromere regions. Prior to the onset of chromosome segregation, sister kinetochores (i.e., a pair of kinetochores generated on sister chromatids) must interact with microtubules extending from opposite spindle poles; this state is called sister kinetochore bi-orientation or amphitelic kinetochores–microtubule attachment. Failure in this process would lead to chromosome missegregation and aneuploidy, which is a hallmark of several human diseases such as cancer and congenital disorders (Hassold and Hunt 2001; Rajagopalan and Lengauer 2004). In this review article, I discuss recent advances in researching the mechanisms ensuring sister kinetochore bi-orientation on the mitotic spindle. In this context, the following topics will also be touched upon briefly—kinetochore composition and assembly (Cleveland et al. 2003; Maiato et al. 2004a; Westermann et al. 2007; Cheeseman and Desai 2008), the spindle assembly checkpoint (Musacchio and Salmon 2007; Burke and Stukenberg 2008), dynamics of spindle microtubules (Gadde and Heald 2004; Kline-Smith and Walczak 2004; Howard and Hyman 2007), and chromosome bi-orientation in meiosis (Hauf and Watanabe 2004; Marston and Amon 2004). However, these topics have been reviewed in more detail in the indicated references.

Initial kinetochores–microtubule interaction; lateral attachment evolving to end-on coupling

In trapeze acrobatics, performers first grab a wired swing with their hands before jumping into the air. Similarly, chromosomes must ensure a secure first contact with microtubules, and this happens in the following cell cycle

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T. U. Tanaka (✉)
Wellcome Trust Centre for Gene Regulation & Expression,
College of Life Sciences, University of Dundee,
Dundee, Scotland
e-mail: t.tanaka@lifesci.dundee.ac.uk

stage. In metazoan cells, microtubule-organizing centers (MTOCs), called centrosomes, locate outside of the nucleus (Azimzadeh and Bornens 2007); therefore, microtubules extending from MTOCs can interact with kinetochores only after the nuclear envelope is broken down at the beginning of mitosis (prometaphase; this is known as “open” mitosis; Sazer 2005). On the other hand, in many single-cell eukaryotes including budding yeast, the nuclear envelope is not broken down during mitosis (called “closed” mitosis; Winey and O’Toole 2001). In budding yeast, kinetochores are connected to MTOCs (called spindle pole bodies) by microtubules even during G1 phase (Winey and O’Toole 2001; Dorn et al. 2005) and it was actually thought that kinetochores–microtubule interaction might be maintained throughout the cell cycle and never be interrupted. However, it was recently revealed that, upon centromere DNA replication, kinetochores are transiently disassembled, causing centromere detachment from microtubules for 1–2 min (Kitamura et al. 2007). Subsequently, kinetochores are reassembled and interact with microtubules again.

In yeast and metazoan cells, how do kinetochores initially interact with microtubules? Kinetochores initially attach to the lateral side of a single microtubule that extends from either spindle pole (where an MTOC is present; Fig. 1, step 1; Hayden et al. 1990; Rieder and Alexander 1990; Tanaka et al. 2005a). The lateral microtubule surface, known as the lattice, provides much larger contact surface, compared with microtubule tips, thus, contributing to an efficient first encounter with kinetochores. The capture of microtubule lattice by kinetochores was initially discovered in newt lung cells (Hayden et al. 1990; Rieder and Alexander 1990) and subsequently found in budding yeast and fission yeast (Tanaka et al. 2005a; Franco et al. 2007; Gachet et al. 2008); therefore, this mode of the capture is widely conserved among eukaryotic cells.

Prior to the initial interaction between microtubules and kinetochores, microtubules repeatedly grow and shrink in various directions, thus, in effect searching for kinetochores (Kirschner and Mitchison 1986). However, the initial encounter happens more efficiently than is likely to be explained by a random search-and-capture process (Wollman et al. 2005). In the *Xenopus* egg extract system, this efficiency might be explained by the presence of a concentration gradient of RanGTP and its associated proteins, around the chromosomes, which facilitates microtubule extension towards chromosomes (Carazo-Salas and Karsenti 2003; Caudron et al. 2005). Moreover, in yeast, fly, and vertebrate cells, microtubules extend not only from spindle poles but also from kinetochores (Khodjakov et al. 2003; Maiato et al. 2004b; Rieder 2005; our unpublished data). Such kinetochore-derived microtubules subsequently interact with pole-derived microtubules and seem to help in recruiting kinetochores

onto the lattice of pole-derived microtubules, at least in some occasions.

Once bound to the microtubule lattice, kinetochores are transported towards a spindle pole along the microtubule (Fig. 1, step 2, sliding). Poleward kinetochore transport is especially crucial when kinetochores are located far away from the mitotic spindle. Kinetochore sliding along a microtubule is promoted by minus end-directed motor proteins, dynein in vertebrate cells (King et al. 2000; Yang et al. 2007), and Kar3, a kinesin-14 family member, in budding yeast (Tanaka et al. 2005a, 2007). Dynein is a processive, and Kar3 is a nonprocessive motor protein, meaning that the motor–microtubule interaction is maintained or interrupted, respectively, after each ATPase cycle (Endow 2003). This explains the rapid kinetochore sliding (10–50 $\mu\text{m}/\text{min}$) in vertebrate cells (Hayden et al. 1990; Rieder and Alexander 1990) and slow/intermittent sliding (1–1.5 $\mu\text{m}/\text{min}$) in yeast (Tanaka et al. 2005a, 2007). Dynein localizes only outside of nuclei in yeast (Hildebrandt and Hoyt 2000); presumably, upon the evolution of open mitosis, metazoans acquired the ability to use dynein in functions that had previously been exclusively nuclear.

Microtubules still maintain their dynamic nature, growing, and shrinking at their plus ends distal to the spindle pole, even while kinetochores are transported along their lattice (Hayden et al. 1990; Rieder and Alexander 1990; Tanaka et al. 2005a). This may pose a challenge to budding yeast, because the speed of microtubule shrinkage (2.5–3 $\mu\text{m}/\text{min}$) exceeds the velocity of kinetochore sliding (Tanaka et al. 2005a). Shrinking microtubule plus ends indeed often catch up with sliding kinetochores, which potentially could cause their subsequent release from the plus ends. However, such release hardly ever occurs because, when the microtubule plus ends reach kinetochores, either of the following two options is taken (Tanaka et al. 2007): (1) microtubules show regrowth (rescue) or (2) kinetochores become tethered at microtubule plus ends and continue to be pulled further towards a spindle pole as microtubules shrink (Fig. 1, step 2, end-on pulling). Kinetochore attachment at the microtubule end is more stable than its lattice association (Tanaka et al. 2007), and, therefore, ideal to resist tension when bi-orientation is subsequently established (see “Geometry- and tension-dependent mechanisms promoting bi-orientation” section).

Once kinetochores attach to the microtubule ends, they cannot revert to lateral attachment to the same microtubules (Tanaka et al. 2007). However, when end-on attached kinetochores reach a spindle pole in vertebrate cells, they can slide along the surface of other microtubules that are already attached to bi-oriented kinetochores, while maintaining their original end-on attachment (Kapoor et al. 2006). This sliding takes place away from a spindle pole, driven by the CENP-E motor protein, and

helps kinetochores to align in the middle of the metaphase spindle.

Interface of kinetochore microtubule interaction: the Ndc80 and Dam1 complexes

Kinetochores are large protein complexes, consisting of dozens of protein components (reviewed in Cleveland et al. 2003; Maiato et al. 2004a; Westermann et al. 2007; Cheeseman and Desai 2008). Among them, which components make a direct contact with microtubules? Recent studies have revealed that two components, the Ndc80 and Dam1 complexes, play central roles in making this contact.

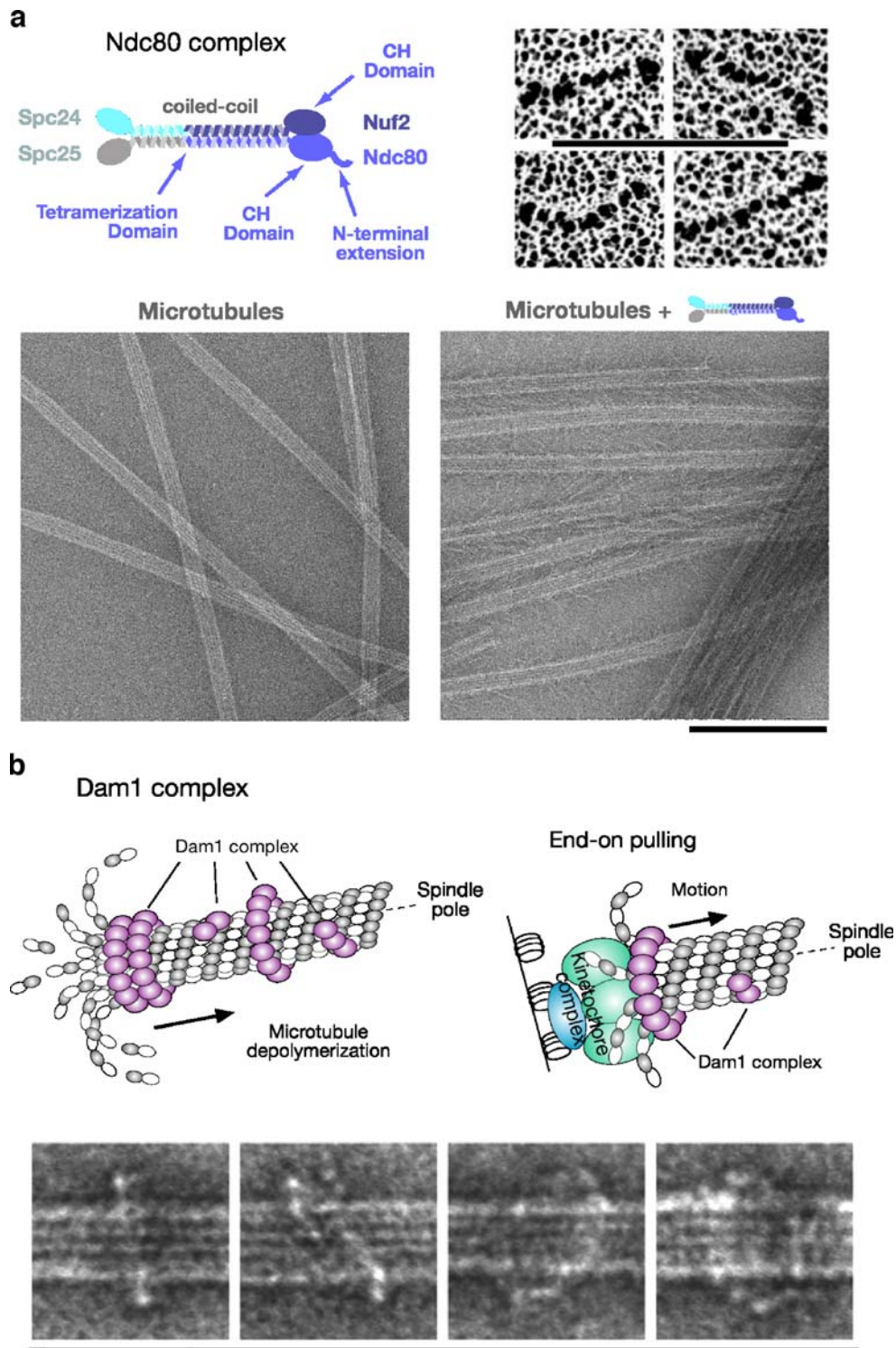
The Ndc80 complex is an outer kinetochore component, structurally conserved from yeast to vertebrates, and the depletion or inactivation of this complex causes severe defects in kinetochores–microtubule interaction (reviewed in Ciferri et al. 2007; Cheeseman and Desai 2008). The Ndc80 complex is composed of four proteins: Ndc80 (also called Hec1 in mammals), Nuf2, Spc24, and Spc25 (Fig. 2a). Ndc80–Nuf2 and Spc24–Spc25 form heterodimers with a globular domain at the end of a coiled-coil shaft (Ciferri et al. 2005; Wei et al. 2005; Wei et al. 2006). The two heterodimers are held together by interaction of their coiled-coil shafts, making a heterotetrameric rod structure with globular domains at both ends. The Spc24–Spc25 globular domain is oriented towards the inner kinetochore and the rod structure projects outwards to microtubules (DeLuca et al. 2006).

Importantly, recent biochemical analyses and electron microscopy showed that the Ndc80–Nuf2 globular domain directly interacts with the microtubule lattice, without showing preference for microtubule ends (Fig. 2a; Cheeseman et al. 2006; Wei et al. 2007). Consistent with this, the Ndc80 complex has a crucial role in kinetochore association with the microtubule lattice in vivo in budding yeast (Fig. 1, step 1; Tanaka et al. 2005a). The crystal structure of the Ndc80–Nuf2 globular domain revealed that each of the Ndc80 and Nuf2 polypeptides is folded as a calponin homology (CH) domain (Wei et al. 2007; Ciferri et al. 2008). Positively charged residues in the CH domains are important for microtubule lattice binding (Ciferri et al. 2008). Another microtubule-associated protein EB1 also folds as a CH domain (Hayashi and Ikura 2003), which is, therefore, a commonly used structure for microtubule association. In Ndc80, an unstructured N-terminal 80- to 100-residue basic region protrudes from the CH domain and phosphorylation of this peptide by Aurora B kinase has a crucial role in regulating kinetochores–microtubule interaction (Cheeseman et al. 2006; DeLuca et al. 2006; Ciferri et al. 2008; see “Regulators promoting bi-orientation: Aurora B/Ipl1 kinase and more” section).

The Ndc80 complex is associated with other kinetochore components, KNL1 (Spc105/Spc7 budding and fission yeast, respectively), and the Mis12 complex (Liu et al. 2005; Cheeseman et al. 2006). This interacting protein set, called the KMN network, shows higher affinity for microtubules than the Ndc80 complex alone (Cheeseman et al. 2006). In particular, KNL1 seems to make an additional microtubule-binding interface (Cheeseman et al. 2006; Kiyomitsu et al. 2007).

As discussed above, kinetochores initially interact with the microtubule lattice and subsequently attach to the plus ends of microtubules. To maintain kinetochore association with dynamic microtubule plus ends, which molecules are involved in their interface? In yeast cells, the Dam1 complex has a crucial role in this process. The Dam1 complex, also called DASH or DDD, is composed of ten proteins and has been identified in budding and fission yeasts (reviewed in Westermann et al. 2007). The Dam1 complex locates along microtubules, but not at kinetochores during their microtubule lattice association (Kitamura et al. 2007; Tanaka et al. 2007). Subsequently this complex is loaded onto kinetochores and plays an important role, perhaps in association with the Ndc80 complex, in tethering them at microtubule plus ends (Janke et al. 2002; Shang et al. 2003; Wong et al. 2007) as well as in pulling them poleward as microtubules depolymerize (Fig. 1, step 2, end-on pulling; Fig. 2b; Franco et al. 2007; Tanaka et al. 2007; Gachet et al. 2008). Biochemical reconstitution revealed that about 16 Dam1 complexes oligomerize and form a ring that encircles a microtubule (Fig. 2b; Miranda et al. 2005; Westermann et al. 2005; Wang et al. 2007). Separate studies suggest that the Dam1 complex can also be present on a microtubule without

Fig. 2 The Ndc80 complex and the Dam1 complex. **a** The Ndc80 complex. Diagram shows four components and defined domains of the complex (*top, left*). Rotary shadowing electron micrographs show rod-like structure of the complex (*top, right*; scale bar 100 nm; reprinted from Wei et al. 2005, with permission; Copyright © 2005 The National Academy of Sciences of the USA). Negatively stained microtubules in the presence (*bottom right*) and absence (*bottom left*) of the Ndc80 complex (reprinted from Cheeseman et al. 2006, with permission; Copyright © 2006 Elsevier). The Ndc80 complex forms angled rod-like projections on the microtubule lattice. **b** The Dam1 complex. The Dam1 complexes are present in vitro as a ring encircling a microtubule (Miranda et al. 2005; Westermann et al. 2005; Wang et al. 2007) but also as an oligomer that does not form a ring (Gestaut et al. 2008; Grishchuk et al. 2008). Both forms could accumulate at the microtubule plus end during the outward curling of protofilaments that accompanies depolymerization (*top, left*); this accumulation was observed in vitro (Westermann et al. 2006; Gestaut et al. 2008; Grishchuk et al. 2008) and in vivo (Tanaka et al. 2007). The Dam1 complex has a crucial role in tethering kinetochores at microtubule ends and in converting microtubule depolymerization into kinetochore pulling force (*top, right*; Asbury et al. 2006; Westermann et al. 2006; Tanaka et al. 2007). Electron micrographs of negatively stained microtubules in the presence of the Dam1 complexes (*bottom*, scale bar 50 nm; reprinted from Miranda et al. 2005, with permission; Copyright © 2005 Nature Publishing Group)



forming a ring (Gestaut et al. 2008; Grishchuk et al. 2008). Both forms can track and accumulate at the plus ends of depolymerizing microtubules in vitro (Asbury et al. 2006; Westermann et al. 2006; Gestaut et al. 2008; Grishchuk et al. 2008), as the Dam1 complex indeed does so in vivo (Tanaka et al. 2007), though whether as rings, independent complexes or both is not yet known.

During microtubule depolymerization, protofilaments splay out at the plus ends. In vitro reconstitution and mathematical models suggest that such protofilament curling produces a force sufficient to move chromosomes towards spindle pole (Grishchuk et al. 2005; Liu and Onuchic 2006; Efremov et al. 2007). It is suggested that the Dam1 complex is required to convert microtubule depoly-

merization to a kinetochore pulling force (Asbury et al. 2006; Westermann et al. 2006; Tanaka et al. 2007). In particular, if the Dam1 complex forms a ring in vivo as well as in vitro, such a ring (whose inner diameter is slightly larger than the microtubule diameter; Miranda et al. 2005; Westermann et al. 2005; Wang et al. 2007) will be pushed poleward by protofilament curling, making an ideal device for end-on pulling of kinetochores (Fig. 2b). This function of the Dam1 complex is important for chromosome motion in prometaphase (Fig. 1, step 2, end-on pulling; Kitamura et al. 2007; Tanaka et al. 2007); presumably, the complex plays a similar role in anaphase A, where kinetochores move towards a spindle pole again by microtubule end-on pulling (Fig. 1, step 5). In addition, the complex may also play a role in tension-coupled chromosome oscillation during metaphase, as suggested by an in vitro reconstitution study (Franck et al. 2007).

Although the Dam1 complex has essential roles in kinetochore association with the end of microtubules in budding yeast, convincing orthologs of Dam1 components have not been identified in metazoa (Meraldi et al. 2006). How can this be reconciled? One possibility is that functional counterparts of the Dam1 complex exist in metazoa, albeit with little homology in amino acid sequences; Ska1/2, Cep57, and Bod1 are such candidates (Hanisch et al. 2006; Emanuele and Stukenberg 2007; Porter and Swedlow 2007), although their functional similarity to the Dam1 complex must be studied further. Alternatively, the role of the Dam1 complex may be more important in organisms such as budding yeast, in which a single kinetochore attaches to a single microtubule in metaphase (Winey et al. 1995). Thus, functional counterparts of the Dam1 complex may not be required in metazoan cells, which have several microtubules per kinetochore and in which components such as the KMN network (see above) may be sufficient for kinetochore association with the ends of the majority of microtubules, even if some microtubules may detach. Consistent with this notion, fission yeast (but not budding yeast), where two–four microtubules attach to a single kinetochore (Ding et al. 1993), can still proliferate in the absence of the Dam1 complex, albeit with frequent chromosome missegregation (Liu et al. 2005; Sanchez-Perez et al. 2005). Requirement of the Dam1 complex, and any possible counterparts, for cell proliferation may be also correlated with their ability to form a ring encircling a microtubule (see above). Intriguingly, budding yeast has a sufficient number of the Dam1 complexes to form a ring at each kinetochore microtubule (Joglekar et al. 2006), but fission yeast has fewer (Joglekar et al. 2008). Electron tomography of vertebrate cells also suggested that microtubule ends are embedded in a fibrous network rather than in ring structures, within kinetochore outer plates in metaphase (Dong et al. 2007).

Geometry- and tension-dependent mechanisms promoting bi-orientation

After kinetochores are transported polewards (Fig. 1, step 2), each sister kinetochore eventually attaches to microtubules that extend from opposite spindle poles (bi-orientation; Fig. 1, step 4). To achieve bi-orientation, wrong orientations of kinetochore microtubule attachment, e.g., sister kinetochores attaching to microtubules from the same spindle pole (syntelic attachment; Fig. 3), must be either avoided or corrected before anaphase onset. It has been thought that two kinds of mechanism could promote this process—a geometry-dependent mechanism and a tension-dependent mechanism (Ault and Rieder 1992; Tanaka et al. 2005b).

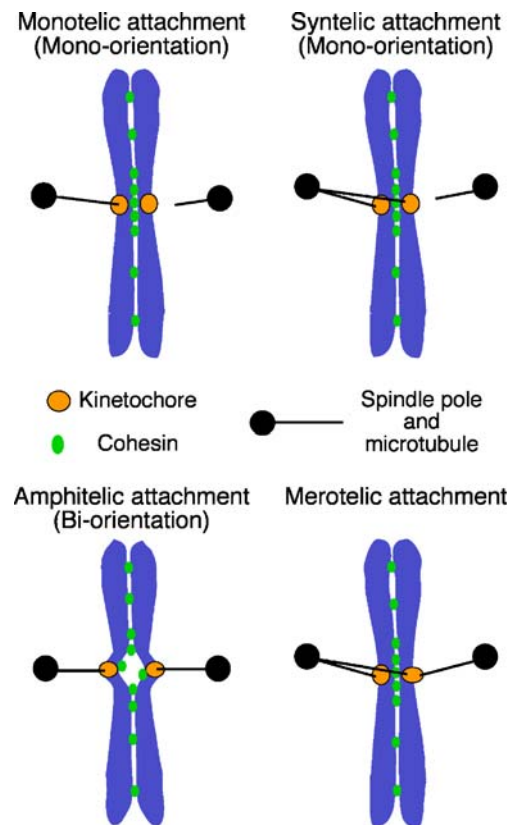


Fig. 3 Modes of kinetochore–microtubule interactions. *Monotelic attachment*—one of the sister kinetochores attaches to microtubules whereas the other does not attach to any microtubules. *Syntelic attachment*—both sister kinetochores attach to microtubules extending from one spindle pole. As a result of monotelic or syntelic attachment, sister kinetochores “mono-orient”; that is, they are connected to only one spindle pole directly or indirectly. *Amphitelic attachment*—each sister kinetochore attaches to microtubules extending from opposite spindle poles. As a result of amphitelic attachment, sister kinetochores “bi-orient”; that is, they are connected to the opposite spindle poles. *Merotelic attachment*—one sister kinetochore simultaneously attaches to microtubules extending from both spindle poles. The figure is adapted from a figure in Tanaka et al. (2005b)

The geometry-dependent mechanism relies on a back-to-back position of sister kinetochores mainly due to cohesion between sister centromeres. When one kinetochore attaches to microtubules from one spindle pole (monotelic attachment; Fig. 3), the constraint in geometry makes the other face the opposite direction, allowing association with microtubules only from the opposite pole. Thus, wrong orientations of kinetochore–microtubule attachment would be discouraged by this mechanism. However, if wrong orientations are formed, the geometry mechanism can no longer correct them.

By contrast, the tension mechanism is important in correcting wrong orientation of kinetochore–microtubule attachment, relying on tension applied on this attachment (Ault and Rieder 1992; Tanaka et al. 2005b). Such error correction was first discovered in meiosis I, where two homologous kinetochores must bi-orient on the spindle. They are connected via chiasmata and able to change their geometry flexibly. Here, kinetochores and spindle poles are repeatedly connected and disconnected by microtubules until bi-orientation is established (Nicklas 1997). Using microneedle manipulation in grasshopper spermatocytes, Nicklas and colleague showed that tension applied on chromosomes leads to stabilization of kinetochore–spindle pole connections (Nicklas and Koch 1969; Nicklas 1997).

In contrast to meiosis I, both geometry- and tension-mechanisms may work in mitosis; however, the relative contribution of the two mechanisms in promoting bi-orientation in mitosis has been an issue of debate (Ault and Rieder 1992). To address this issue, an unreplicated circular minichromosome with two centromeres was engineered and its behavior was observed in budding yeast (Dewar et al. 2004). On this minichromosome, two centromeres would lack back-to-back geometry as they are not sisters born by DNA replication, but tension should be generated across them by intercentromere chromatin, if two centromeres bi-orient on the spindle. Such a minichromosome always and efficiently bi-oriented, suggesting that tension across its two kinetochores is sufficient to promote bi-orientation (Dewar et al. 2004). The corollary of this observation is that in normal sister chromatids, kinetochore–spindle pole connections change their orientation repeatedly until, upon bi-orientation, cohesion between sister kinetochores provides resistance and consequently tension, which stabilizes the connections (Fig. 1, steps 3–4).

However, the above observation does not exclude a redundant role for geometry in facilitating bi-orientation in mitosis (Indjeian and Murray 2007). Cohesion between sister kinetochores is thought to be important for sister kinetochore geometry. Thus, it is likely that cohesion has important roles in both geometry- and tension-dependent mechanisms for bi-orientation. Cohesins are required for sister chromatid cohesion (Nasmyth 2002) and their depletion

indeed leads to extensive defects in bi-orientation (Tanaka et al. 2000; Sonoda et al. 2001; Dewar et al. 2004; Vagnarelli et al. 2004). In particular, recent studies characterized the relevant role of cohesins at pericentric regions in yeast (Eckert et al. 2007; Ocampo-Hafalla et al. 2007; Yeh et al. 2008). The geometry of sister kinetochores may also be facilitated by their nature such that, once forming a particular geometry, they ‘memorize’ and tend to maintain such geometry (Loncarek et al. 2007). However, after syntelic attachment is sustained for some time, sister kinetochores remain juxtaposed due to this nature and in order to restore their back-to-back geometry, microtubules must pull them towards opposite spindle poles (Loncarek et al. 2007).

In contrast to budding yeast, multiple microtubules attach to a single kinetochore in fission yeast and metazoan cells (McDonald et al. 1992; Ding et al. 1993). In this situation, errors could happen in such a way that a single kinetochore becomes attached to microtubules from the opposite spindle poles (merotelic attachment; Fig. 3). How do cells avoid and/or correct this type of errors? Merotelic attachments are probably discouraged by geometry-dependent mechanisms. For example, the Psc1/Mde4 complex is a proposed clamp to ensure this geometry in fission yeast kinetochores (Gregan et al. 2007). However, if merotelic attachments are unavoidably formed, some of them still seem to be corrected prior to anaphase onset (see “Regulators promoting bi-orientation: Aurora B/Ipl1 kinase and more” section; Cimini et al. 2003; Cimini et al. 2006). Even if merotelic attachments remain until anaphase, the imbalance between forces applied on sister kinetochores often results in their proper segregation (Cimini et al. 2004).

Regulators promoting bi-orientation: Aurora B/Ipl1 kinase and more

In addition to proteins necessary for the kinetochore–spindle pole connections by microtubules and for sister chromatid cohesion, what factors are required to promote sister kinetochore bi-orientation? Aurora B (called Ipl1 in budding yeast) is an evolutionarily conserved serine/threonine protein kinase and has essential roles in promoting bi-orientation (reviewed in Tanaka et al. 2005b; Ruchaud et al. 2007). In fact, inhibition or mutants of Aurora B/Ipl1 show extensive chromosome missegregation in metazoan and yeast cells.

In *ipl1* mutants, the kinetochore–spindle pole connections are present but their orientation is specifically defective (Biggins and Murray 2001; He et al. 2001; Tanaka et al. 2002). In the *ipl1* mutant, an unreplicated circular minichromosome with two centromeres (see above) often failed to bi-orient, suggesting that Ipl1 could facilitate bi-orientation by a tension-dependent mechanism (Dewar

et al. 2004). Moreover, in this mutant, centromeres failed to change their spindle pole association in the absence of tension (Tanaka et al. 2002; Dewar et al. 2004). It was therefore suggested that Ipl1 kinase promotes turnover of kinetochore–spindle pole microtubule connections and eliminates those that do not generate tension between sister kinetochores (Fig. 4). Aurora B has a similar role in mammalian cells, as they accumulate syntelic kinetochore–microtubule attachments when this kinase is inhibited (Hauf et al. 2003; Lampson et al. 2004).

Aurora B/Ipl1 localizes at kinetochores in prometaphase and metaphase and seems to promote turnover of kinetochore–microtubules attachments by phosphorylating kinetochore components. Crucial substrates include the Dam1 complex in budding yeast (Cheeseman et al. 2002; Zhang et al. 2005) and the Ndc80 complex (Cheeseman et al. 2006; DeLuca et al. 2006; Ciferri et al. 2008) and MCAK (Andrews et al. 2004; Lan et al. 2004; Ohi et al. 2004; Knowlton et al. 2006; Zhang et al. 2007) in mammalian cells. Phosphorylation of Dam1 is clustered at its C terminus and this region is important for ring formation and microtubule interaction of the Dam1 complex in vitro (Cheeseman et al. 2002; Wang et al. 2007). On the other hand, phosphorylation of Ndc80 is clustered at its N-

terminal basic region and reduces affinity of the Ndc80 complex for microtubules in vitro (Cheeseman et al. 2006; Ciferri et al. 2008). Moreover, *dam1* and *ndc80* mutants mimicking constitutive dephosphorylation show defects in bi-orientation in vivo (Cheeseman et al. 2002; DeLuca et al. 2006). Furthermore, the microtubule depolymerizing activity of MCAK, regulated by Aurora B, might be important to remove syntelic and merotelic attachments (Andrews et al. 2004; Lan et al. 2004; Ohi et al. 2004; Knowlton et al. 2006; Zhang et al. 2007).

In metazoan cells where multiple microtubules attach to a single kinetochore, Aurora B may have a more complex role in promoting bi-orientation. For example, inhibition of Aurora B leads to not only syntelic attachment but also merotelic attachment (Cimini et al. 2006; Knowlton et al. 2006; see “Geometry- and tension-dependent mechanisms promoting bi-orientation” section). Aurora B (together with Polo kinase) also promotes resolution of sister chromatids (Losada et al. 2002), which may facilitate bi-orientation, in addition to turnover of kinetochore–microtubule attachment promoted by this kinase.

Aurora B/Ipl1 kinases are also known as ‘passenger proteins’ as they relocate from kinetochores to the spindle upon anaphase onset (Ruchaud et al. 2007). This relocation, regulated by Cdc14 phosphatase in yeast (Pereira and Schiebel 2003) and cyclin B destruction in fly (Parry et al. 2003), is probably important to stop the turnover of kinetochore–spindle pole connections during anaphase, when tension on kinetochores is much reduced. In fact, when this relocation of Aurora B/Ipl1 is inhibited, kinetochores do continuously re-orient on the anaphase spindle (Parry et al. 2003).

Mps1 is another evolutionarily conserved protein kinase, which is required for the spindle assembly checkpoint and, in some organisms, for duplication of MTOCs of the mitotic spindle (reviewed in Winey and Huneycutt 2002). Separately from these functions, however, Mps1 has an important role in chromosome segregation, especially in sister kinetochore bi-orientation (Jones et al. 2005; Maure et al. 2007). Similarly to Aurora B/Ipl1, Mps1 promotes turnover of kinetochore–microtubule attachment that does not generate tension (Maure et al. 2007). In humans, Mps1 regulates Aurora B kinase activity by phosphorylating Borealin (also called Dasra B) that binds Aurora B (Jelluma et al. 2008); this explains the role of Mps1 as an upstream regulator of Aurora B. Budding yeast, however, does not have an ortholog of Borealin, and Mps1 and Ipl1 may work in a parallel pathway (Maure et al. 2007).

Once bi-orientation is established and tension is applied on kinetochores, turnover of kinetochore–spindle pole connections must stop (Pearson et al. 2004; Tanaka et al. 2005b); otherwise, bi-orientation would never be maintained. For this, sensing tension is of central importance,

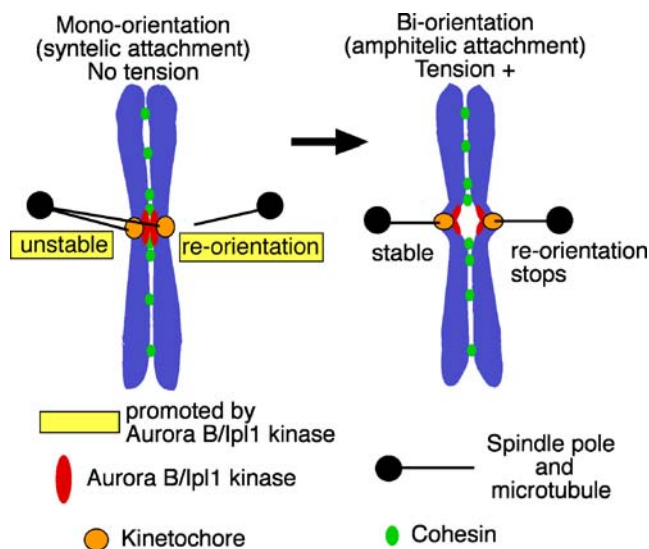


Fig. 4 How Aurora B/Ipl1 facilitates sister kinetochore bi-orientation. The Aurora B/Ipl1 kinase facilitates bi-orientation by promoting the re-orientation of kinetochore–spindle pole connections in a tension-dependent manner (Tanaka et al. 2002; Dewar et al. 2004). Because syntelic attachment does not generate tension on kinetochore-to-pole connections, Aurora B/Ipl1 promotes re-orientation of these connections (by phosphorylating kinetochore components; see main text). When an amphitelic attachment is established, tension is applied on kinetochore-to-pole connections, and as a result, Aurora B/Ipl1 stops promoting their re-orientation, which causes preferential selection of the amphitelic attachment. The figure is adapted from a figure in Tanaka et al. (2005b)

but which component works as a tension sensor? Bir1 and Sli15 (Survivin and INCENP in metazoa) are binding partners of Ipl1 in yeast and regulate its kinase activity (reviewed in Ruchaud et al. 2007). Bir1 and Sli15 form a subcomplex bridging between a microtubule and a kinetochore and, thus, ideally positioned to sense tension (Sandall et al. 2006); they may regulate Ipl1 activity accordingly although this remains to be demonstrated. On the other hand, in the *Xenopus* egg extract system, the kinase activity of Aurora B, locating at inner centromeres, may be enhanced by proximity to microtubules, only during syntelic and merotelic attachment (Ohi et al. 2003; Rosasco-Nitcher et al. 2008). In mammals, another candidate for a tension sensor might be PICH, a Snf2 family member, which shows a unique thread-like localization between bi-oriented sister kinetochores (Baumann et al. 2007). In an alternative model, when tension is applied, kinetochore substrates may be sequestered from Aurora B that locates at inner centromeres in animal cells; this may lead to dephosphorylation of kinetochore substrates (Tanaka et al. 2002; Andrews et al. 2004). However, in budding yeast, such a delocalization between kinetochores and Ipl1 may not be easily observed due to resolution constraints (Buvelot et al. 2003; Tanaka et al. 2005b).

Bub1 and Sgo proteins (Sgo1 in budding yeast and Sgo2 in fission yeast) are required to ensure bi-orientation in yeast (Asakawa et al. 2005; Fernius and Hardwick 2007; Kawashima et al. 2007; Vanoosthuyse et al. 2007) and in mammals (Meraldi and Sorger 2005). Nonetheless, in their absence, yeast cells are still able to proliferate, albeit with increased rates of chromosome missegregation, suggesting a role for them in “fine tuning” of bi-orientation (contrasting with the essential roles of Aurora B/Ipl1 and Mps1 kinases). It is suggested that Bub1 is required for Sgo recruitment at kinetochores, which in turn contributes to full-scale Aurora B loading there (Fernius and Hardwick 2007; Kawashima et al. 2007; Vanoosthuyse et al. 2007).

Bi-orientation and spindle assembly checkpoint: convoluted relation

The spindle assembly checkpoint is a surveillance mechanism that delays anaphase onset if kinetochores fail to attach to microtubules, or if sister kinetochores fail to bi-orient on the spindle (reviewed in Musacchio and Salmon 2007; Burke and Stukenberg 2008). Such failure is sensed at kinetochores, signals via Mad and Bub proteins and eventually inhibits Cdc20, an activator of the anaphase-promoting complex. The spindle assembly checkpoint is distinct from the bi-orientation-promoting mechanisms discussed above (“Geometry- and tension-dependent mech-

anisms promoting bi-orientation” and “Regulators promoting bi-orientation: Aurora B/Ipl1 kinase and more” sections); the former one does not promote bi-orientation by itself but rather earns time for the latter one to promote bi-orientation. Moreover, in yeast, the spindle assembly checkpoint is not required for proper chromosome segregation during normal, undisturbed cell cycles (Warren et al. 2002), in contrast to mechanisms promoting bi-orientation. This suggests that yeast cells normally have enough time to establish bi-orientation without relying on checkpoint-dependent delay of anaphase onset. By contrast, in mammals, the spindle assembly checkpoint is crucial for proper chromosome segregation even during normal cell cycles (e.g., Dobles et al. 2000). Nonetheless, in mammals, defects in the checkpoint can be distinguished from defects in bi-orientation-promoting mechanisms as follows: when metaphase is prolonged, for example, using a proteasome inhibitor, bi-orientation is restored in cells with a checkpoint defect, but not in cells with a defect in bi-orientation-promoting mechanisms (e.g., Jelluma et al. 2008).

Although the two mechanisms are clearly distinct, they cooperate with each other to establish bi-orientation; checkpoint buys time for bi-orientation-promoting mechanisms to work. However, their cooperation is not unilateral. In some cases, due to bi-orientation-promoting mechanisms, the spindle assembly checkpoint remains active. For example, when no tension is applied on kinetochores, Aurora B/Ipl1 promotes turnover of kinetochore–microtubule attachment, thus, generating unattached kinetochores which keeps the checkpoint active (Tanaka et al. 2002; Hauf et al. 2003; Pinsky et al. 2006). Mps1 and Sgo may be involved in maintaining checkpoint activity by a similar mechanism in the absence of tension on kinetochores (Dorer et al. 2005; Indjeian et al. 2005). Thus, defects in bi-orientation are sensed by the checkpoint, partly through generation of unattached kinetochores.

However, independently of this process, an active spindle assembly checkpoint seems to require Aurora B/Ipl1 (Ditchfield et al. 2003; Morrow et al. 2005; King et al. 2007) and Mps1 kinases (see below). For example, when tension is not applied on kinetochores, Mad3 phosphorylation by Ipl1 becomes essential for checkpoint activity, but not for establishment of bi-orientation (therefore, presumably, not for turnover of kinetochore–microtubule attachment; King et al. 2007). Moreover, when kinetochores fail to attach to microtubules (e.g., when microtubules are depolymerized by nocodazole), Mps1 is crucial for activating the checkpoint even if there is no kinetochore–microtubule attachment to be turned over (Weiss and Winey 1996). Thus, the spindle assembly checkpoint and mechanisms promoting bi-orientation are related in a convoluted way, which ensures their close cooperation to achieve bi-orientation.

Conclusions and perspectives

Establishing sister kinetochore bi-orientation is a pivotal process for ensuring equal segregation of the genetic information into daughter cells upon cell division. To understand this process, many pertinent questions remain to be answered. For example, how do kinetochores and microtubules efficiently encounter each other and what are the roles of RanGTP gradients, kinetochore-derived microtubules and other factors in this process? How is the kinetochore-microtubule interaction converted from lateral to end-on and how are kinetochores associated with the end of dynamic microtubules; in particular, how are possible Dam1-like-regulators in metazoa and the KMN network involved in this process? How are the merotelic attachments corrected to proper bi-orientation? How does phosphorylation of kinetochore components by Aurora B/Ipl1 facilitate turnover of kinetochore–spindle pole connection? How is the tension on this connection sensed, leading to cessation of this turnover accordingly? How is the bi-orientation-promoting mechanism interlinked with the spindle assembly checkpoint, in particular, do they share tension-sensing mechanisms while using different outputs? The combined efforts in biochemical reconstitution, structural study, genetics, and cell biology will be necessary to advance research in this field.

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