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Kinetochores–microtubule interactions: steps towards bi-orientation

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Eukaryotic cells segregate their chromosomes accurately to opposite poles during mitosis, which is necessary for maintenance of their genetic integrity. This process mainly relies on the forces generated by kinetochores–microtubule (KT–MT) attachment. During prometaphase, the KT initially interacts with a single MT extending from a spindle pole and then moves towards a spindle pole. Subsequently, MTs from the other spindle pole also interact with the KT. Eventually, one sister KT becomes attached to MTs from one pole while the other sister to those from the other pole (sister KT bi-orientation). If sister KTs interact with MTs with aberrant orientation, this must be corrected to attain proper bi-orientation (error correction) before the anaphase is initiated. Here, I discuss how KTs initially interact with MTs and how this interaction develops into bi-orientation; both processes are fundamentally crucial for proper chromosome segregation in the subsequent anaphase.

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Introduction

The proper segregation of sister chromatids to opposite poles of the cell during mitosis is crucial for cell proliferation. This topic has important medical relevance because chromosome mis-segregation can have causative functions in a variety of human diseases such as cancers and congenital disorders, which are characterized by chromosome instability and aneuploidy (Chandhok and Pellman, 2009; Holland and Cleveland, 2009; Thompson *et al.*, 2010).

The segregation of sister chromatids during mitosis mainly depends on the forces generated by microtubules (MTs) that attach to kinetochores (KTs) (Tanaka and Desai, 2008). For proper chromosome segregation, KTs must capture spindle MTs and properly align on the mitotic spindle before ana-

phase onset. Cells undergo these processes in a step-wise manner as follows (Tanaka, 2008):

- (1) The KT initially interacts with the lateral surface of a single MT extending from a spindle pole (spindle-pole MT) (Figure 1, step 1, right).
- (2) Subsequently, the KT is transported along a spindle-pole MT towards a spindle pole (Figure 1, step 2, left).
- (3) The KT is tethered at the plus end of a spindle-pole MT (conversion from the lateral to the end-on attachment) (Figure 1, step 2, right).
- (4) Subsequently, sister KTs attach to MTs extending from opposite spindle poles (sister KT bi-orientation). If sister KTs attach to MTs with aberrant orientation, such errors must be corrected by turnover of the KT–MT attachment (error correction), before anaphase onset (Figure 1, steps 3 and 4).

All these steps are crucial to ensure high-fidelity chromosome segregation in the subsequent anaphase (Figure 1, step 5). In this review article, I discuss the mechanisms ensuring the initial KT–MT interaction and subsequent sister KT bi-orientation on the mitotic spindle.

Note that the following topics are touched upon briefly in the context of this article, but are not the main focus of it: KT composition and assembly (Westermann *et al.*, 2007; Cheeseman and Desai, 2008; Santaguida and Musacchio, 2009); MT dynamics on the mitotic spindle (Walczak and Heald, 2008; Dumont and Mitchison, 2009); chromosome congression on the metaphase plate (Kops *et al.*, 2010); the spindle-assembly checkpoint (SAC) (Musacchio and Salmon, 2007; Burke and Stukenberg, 2008; Khodjakov and Rieder, 2009) and KT–MT interactions in meiosis (Brar and Amon, 2008; Sakuno and Watanabe, 2009). These topics have been reviewed in detail in the indicated references.

Initial KT–MT interaction: mechanisms facilitating their first encounter

KTs must secure their first contact with spindle-pole MTs, and this happens with the following timing in the cell cycle. In metazoan cells, because MT-organizing centres (MTOCs), called centrosomes (Azimzadeh and Bornens, 2007), locate outside of the nucleus in interphase, MTs extending from MTOCs can interact with KTs 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 such as budding yeast, the nuclear envelope is not broken down during mitosis (known as 'closed' mitosis) (Heath, 1980). In budding yeast, KTs are connected to MTOCs (called spindle-pole bodies) by MTs during most of the cell cycle (Winey and O'Toole, 2001).

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However, KTs are transiently disassembled upon centromere DNA replication. This causes centromere detachment from MTs for a few minutes (Kitamura *et al.*, 2007), which is followed by KT reassembly and re-association with MTs.

How do KTs initially interact with MTs in yeast and metazoan cells? KTs initially attach to the lateral side of a single spindle-pole MT (Hayden *et al.*, 1990; Rieder and Alexander, 1990; Tanaka *et al.*, 2005a) (Figure 1, step 1, right). The lateral MT surface, called the lattice, provides a much larger contact surface compared with MT tips, thus contributing to an efficient first encounter with KTs. The capture of the MT lattice by KTs was initially discovered in newt lung cells (Hayden *et al.*, 1990; Rieder and Alexander, 1990) and subsequently found in budding and fission yeast (Tanaka *et al.*, 2005a; Franco *et al.*, 2007; Gachet *et al.*, 2008); therefore, this is an evolutionarily conserved process among eukaryotic cells.

Before the initial interaction between MTs and KTs, MTs undergo repeated growth and shrinkage in various directions, as if they are searching for KTs (Kirschner and Mitchison, 1986). However, the initial encounter is thought to happen more efficiently than is likely to be expected from a random search-and-capture process (Wollman *et al.*, 2005). What then are the mechanisms that could contribute to the efficient KT–MT encounter? In vertebrate cells that undergo open mitosis, a concentration gradient of Ran-GTP is formed around chromosomes and ‘guide’ spindle-pole MTs towards them (Carazo-Salas

and Karsenti, 2003; Caudron *et al.*, 2005; Kalab *et al.*, 2006; O’Connell and Khodjakov, 2007; Clarke and Zhang, 2008). Relevant to this process, CDK11 has been identified as a Ran-GTP-dependent MT stabilization factor (Yokoyama *et al.*, 2008).

The mechanism dependent on the Ran-GTP gradient is indeed effective over a long range ($\sim 20\mu\text{m}$) (Athale *et al.*, 2008). However, Ran-GTP cannot make a substantial gradient over a shorter range (e.g. $< 5\mu\text{m}$) presumably because of its rapid diffusion. Moreover, in cells that undergo closed mitosis, such as yeast, a Ran-GTP gradient is presumably not formed during mitosis, as its concentration is expected to be uniformly high in the nucleus, similar to the interphase nucleus in metazoan cells (Kalab *et al.*, 2006).

Initial KT interaction with spindle-pole MTs is facilitated by KT-derived MTs

Given the argument above, do any additional mechanisms facilitate the initial KT–MT encounter, in particular over a short distance? In yeast, fly and vertebrate cells, it has been revealed that MTs are generated not only at spindle poles but also at KTs (Khodjakov *et al.*, 2003; Maiato *et al.*, 2004; Kitamura *et al.*, 2010). Such KT-derived MTs subsequently interact with MTs extending from spindle poles (i.e. spindle-pole MTs) along their length, facilitating KT loading onto the lattice of spindle-pole MTs (Figure 1, step 1, left). Notably, KTs generate MTs more often when KT interaction with

Step 1. Kinetochore initially interacts with the lateral surface of a microtubule from a spindle pole (often facilitated by a kinetochore-derived microtubule)

Figure 1 Step-wise development of kinetochore-microtubule interactions in eukaryotic cells. The figure shows kinetochore-microtubule (KT-MT) interactions during prometaphase (steps 1–3), metaphase (step 4) and anaphase (step 5). Prometaphase in budding yeast is as defined in Kitamura *et al.* (2007). Step 1: The KT initially interacts with the lateral surface of a single MT (lateral attachment), which extends from one spindle pole (spindle-pole MT) (right) (Hayden *et al.*, 1990; Rieder and Alexander, 1990; Tanaka *et al.*, 2005a). This process is often facilitated by the interaction between a KT-derived MT and a spindle-pole MT (left) (see Figure 2) (Khodjakov *et al.*, 2003; Maiato *et al.*, 2004; Kitamura *et al.*, 2010). It is not yet known whether both or only one of the sister KTs attaches to an MT during lateral attachment. Step 2: Once loaded on a spindle-pole MT, the KT is transported along the MT lateral surface towards a spindle pole (sliding; left) (Hayden *et al.*, 1990; Rieder and Alexander, 1990; Tanaka *et al.*, 2005a). Subsequently, at least in yeast, the KT is tethered at the end of a single spindle-pole MT (end-on attachment) and transported further as the MT shrinks (end-on pulling; right) (Kitamura *et al.*, 2007; Tanaka *et al.*, 2007). Step 3: Following the KT transport towards a spindle pole, both sister KTs could attach to MTs. If both sister KTs attach to MTs from the same spindle pole (syntelic attachment; see Figure 4), the KT-MT attachment must be turned over until proper bi-orientation is established (error correction; see Figure 5) (Nicklas, 1997; Tanaka *et al.*, 2002). Step 4: The turnover of the KT-MT attachment stops once tension is generated across sister KTs upon the establishment of bi-orientation (see Figure 5) (Nicklas, 1997; Tanaka *et al.*, 2002; Dewar *et al.*, 2004). The number of MTs whose plus ends attach to a single KT increases as more tension is applied on metazoan KTs (King and Nicklas, 2000), while only a single MT attaches to each sister KT in budding yeast (Winey *et al.*, 1995). Step 5: Once all sister KTs bi-orient on the spindle, cohesion between sister chromatids is removed, causing sister chromatid segregation to opposite spindle poles during anaphase (Nasmyth, 2002). Sister chromatid separation proceeds along a chromosome arm from the centromere to the telomere in budding yeast (Renshaw *et al.*, 2010). The end-on KT-MT attachment is maintained as the MTs depolymerize and sister KTs are pulled towards the opposite poles during anaphase A, which is followed by the pole-to-pole distance being enlarged during anaphase B.

Figure 2 Kinetochore-derived microtubules in yeast and fly cells. The figure shows generation of MTs at KTs and the roles of KT-derived MTs during early mitosis in yeast (budding yeast) and fly (*Drosophila*) cells. Step 1: In both organisms, the KT can nucleate MTs and generate short MTs with distal plus ends (see discussion in Maiato *et al*, 2004; Kitamura *et al*, 2010); ‘+’ and ‘–’ designate the polarity of relevant MTs. Step 2: In yeast (top), a KT-derived MT extends by tubulin polymerization at the MT plus end, which remains distal to the KT (Kitamura *et al*, 2010). On the other hand, in fly cells (bottom), the plus end of a KT-derived MT is caught on the KT (polarity conversion) and this MT extends by tubulin polymerization at the KT, with the minus end distal to the KT (Khodjakov *et al*, 2003; Maiato *et al*, 2004). Step 3: In both organisms, the KT-derived MT interacts with a spindle-pole MT along their length, which facilitates KT loading onto the lateral surface of the spindle-pole MT (Khodjakov *et al*, 2003; Kitamura *et al*, 2010). Steps 4 and 5: Once the KT is loaded onto the spindle-pole MTs in yeast, already-existing KT-derived MTs shrink and disappear, and the KT cannot generate new MTs (Kitamura *et al*, 2010). On the other hand, in fly cells, KT-derived MTs can extend further and often reach a spindle pole, becoming a part of the spindle (Khodjakov *et al*, 2003; Maiato *et al*, 2004).

spindle-pole MTs is delayed (Kitamura *et al*, 2010); this may occur as KTs become more mature while they remain unattached to the spindle. Thus, the generation of KT-derived MTs seems to help avoid a further delay in the KT encounter with spindle-pole MTs.

Intriguingly, whereas KT-derived MTs have plus ends distal to KTs in yeast cells, their polarity is opposite in *Drosophila* cells and perhaps also in human cells (Khodjakov *et al*, 2003; Maiato *et al*, 2004; Kitamura *et al*, 2010). This discrepancy is reconciled if, in both organisms, short MTs (perhaps 50–500 nm) are generated initially with plus ends distal to KTs (Figure 2, step 1) and if the MT polarity is converted in *Drosophila* (and other metazoan) cells, but not in yeast (Figure 2, step 2). Consistent with this notion, the γ -tubulin complex is present at KTs in vertebrate cells (Mishra *et al*, 2010) and could facilitate MT nucleation at KTs with plus ends distal to KTs. The minus ends of such short MTs are not embedded at KT plates in vertebrate cells (Witt *et al*, 1980) and their distal plus ends might be subsequently captured by KT plates (Figure 2, step 2, bottom). Such polarity conversion could be promoted by plus end-directed motors on KTs in metazoan cells (e.g. CENP-E). On the other hand, no plus end-directed motors are found at the yeast KTs, before sister KT bi-orientation (Tanaka *et al*, 2007), consistent with a lack of the polarity conversion of KT-derived MTs in yeast.

Irrespective of their polarity, KT-derived MTs often interact with spindle-pole MTs along their length, facilitating KT loading onto the spindle-pole MTs (Khodjakov *et al*, 2003; Kitamura *et al*, 2010) (Figure 2, step 3). However, depending on their polarity, the consequences of that interaction differ. When KT-derived MTs have their minus ends distal to KTs, these distal ends often reach spindle poles and become tethered there, thus contributing to the formation of the spindle (Figure 2, steps 4 and 5, bottom) (Khodjakov *et al*, 2003; O’Connell *et al*, 2009). In contrast, in budding yeast, KT-derived MTs have a shorter lifetime and disappear soon after KT loading onto spindle-pole

MTs (Figure 2, steps 4 and 5, top) (Kitamura *et al*, 2010). In budding yeast, Stu2 (the orthologue of vertebrate XMAP215/ch-TOG) localizes at KTs and has a central function in generating MTs at KTs (Kitamura *et al*, 2010).

Conversion from the lateral to end-on KT–MT attachment

Once bound to the MT lattice (lateral attachment), KTs are transported towards a spindle pole along the MT (sliding; Figure 1, step 2). The poleward KT transport is especially crucial when KTs are located far away from the mitotic spindle. KT sliding along an MT 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).

While the KT is associated with the MT lattice, the plus end of the shrinking MT often catches up with the KT, leading to the KT becoming tethered at MT plus end (end-on attachment) and being pulled further towards a spindle pole as the MT shrinks (Tanaka *et al*, 2007) (end-on pulling; Figure 1, step 2).

Is the end-on KT–MT attachment simply a lateral attachment close to a tip of the MT? At least in budding yeast, the end-on attachment shows clearly different features from lateral attachment, as follows (Tanaka *et al*, 2005a, 2007): (1) the speed of poleward KT motion is slower during lateral attachment than during end-on attachment, (2) the speed of MT shrinkage is similar in the presence and absence of the KT on its lateral side, but is slowed down once end-on attachment is established, (3) MT rescue (conversion from MT shrinkage to re-growth) occurs during lateral attachment (KT sliding), but not during end-on attachment (KT end-on pulling) and (4) different regulators are involved during lateral and end-on attachment; that is Kar3 is required for poleward sliding and the Dam1 complex for end-on pulling but not *vice versa* (see below).

Therefore, the end-on attachment is different from the lateral attachment, not only in the KT position but also in their mechanism. The end-on attachment can sustain KT–MT attachment stably (Grishchuk *et al*, 2005; Joglekar *et al*, 2010), presumably more so than lateral attachment; indeed, KT detachment from MTs happens in a small number of cases (~5%) during lateral attachment, but not during end-on attachment (Tanaka *et al*, 2007). Thus, end-on attachment might be ideal to sustain KT–MT interaction when bi-orientation is subsequently established and tension is applied across sister KTs (see below).

In the next two sections, I discuss how the Ndc80 complex, the Dam1 complex and its functional equivalents make interface of KT–MT interaction during the lateral and end-on attachment.

Interface of the KT–MT attachment: the Ndc80 complex

KTs are large protein complexes, consisting of dozens of protein components (Westermann *et al*, 2007; Cheeseman and Desai, 2008; Santaguida and Musacchio, 2009). Among them, the Ndc80 and Dam1 complexes have important functions in making a direct contact with MTs.

The Ndc80 complex is an outer KT component, structurally conserved from yeast to vertebrates, and defects in it lead to severely impaired KT–MT interaction (reviewed in Cheeseman and Desai, 2008; Tanaka and Desai, 2008; Santaguida and Musacchio, 2009). The Ndc80 complex is composed of four proteins (Figure 3A): Ndc80 (also called Hec1 in mammals), Nuf2, Spc24 and Spc25. 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, 2006). Their coiled-coil shafts interact with each other and hold two heterodimers together, making a heterotetrameric rod structure with globular domains at both ends. The Spc24–Spc25 globular domain is oriented towards the inner KT and the rod structure projects the Ndc80–Nuf2 globular domain outwards to MTs (DeLuca *et al*, 2006; Joglekar *et al*, 2009; Wan *et al*, 2009).

The Ndc80–Nuf2 globular domain directly interacts with the MT lattice *in vitro* (Cheeseman *et al*, 2006; Wei *et al*, 2007; Wilson-Kubalek *et al*, 2008). Consistent with this, the Ndc80 complex has a crucial role in KT association with the MT lattice *in vivo* in budding yeast (Figure 1, step 1, right; Tanaka *et al*, 2005a). X-ray crystallography revealed that the Ndc80–Nuf2 globular domain is made of two calponin-homology (CH) domains (Wei *et al*, 2007; Ciferri *et al*, 2008). Positively charged residues in the CH domains are important for MT lattice binding (Ciferri *et al*, 2008). Another MT-associated protein EB1 also folds as a CH domain (Hayashi and Ikura, 2003), which is, therefore, a commonly used structure for MT association. In Ndc80, an unstructured N-terminal 80–100 residue basic region, protruding from the CH domain, is suggested to strengthen the MT association (Guimaraes *et al*, 2008; Miller *et al*, 2008) and inter-complex association (Ciferri *et al*, 2008; Alushin *et al*, 2010).

The Ndc80 complex is linked to the inner KT complex through association between its Spc24–25 globular domain and the Mis12 complex (Cheeseman *et al*, 2006; Maskell *et al*, 2010; Petrovic *et al*, 2010). The Mis12 complex also associates with another KT component KNL1, which is also called

Figure 3 The Ndc80 and Dam1 complexes make interface for kinetochore–microtubule attachment. (A) Structure of the Ndc80 complex, which consists of Ndc80, Nuf2, Spc24 and Spc25 proteins (Wei *et al*, 2007; Ciferri *et al*, 2008). The globular domains of Ndc80 and Nuf2 are folded into calponin-homology (CH) domains. (B) The role of the Ndc80 and Dam1 complexes in the conversion of lateral to end-on KT–MT attachment (see Figure 1, step 2). During the lateral attachment, the Ndc80 complex (shown in red) binds an MT at its CH domains and unstructured N-terminus (Cheeseman *et al*, 2006; Wei *et al*, 2007; Ciferri *et al*, 2008). To convert lateral to end-on attachment, it is probably crucial that the Ndc80 complex becomes associated with the Dam1 complex (shown in green) (Lampert *et al*, 2010; Tien *et al*, 2010; our unpublished results), which localizes at the MT plus end and forms an oligomer and/or a ring encircling the MT (reviewed in Westermann *et al*, 2007; Nogales and Ramey, 2009). The Ndc80 N-terminus is suggested to strengthen the MT association (Guimaraes *et al*, 2008; Miller *et al*, 2008). This region may be also important for inter-complex association (Ciferri *et al*, 2008; Alushin *et al*, 2010). It is not yet clear whether the Ndc80 complexes go through the inside or outside of a Dam1-complex ring, if the ring is formed around an MT.

Blinkin, Spc105 and Spc7 in vertebrates, budding and fission yeast, respectively. This interacting protein set of Ndc80 complex, Mis12 complex and KNL1, is called the KMN network, which shows higher affinity for MTs than the Ndc80 complex alone (Cheeseman *et al*, 2006). In particular, KNL1

seems to make an additional direct MT-binding interface (Cheeseman *et al.*, 2006; Kiyomitsu *et al.*, 2007; Pagliuca *et al.*, 2009; Welburn *et al.*, 2010).

As discussed in the previous section, KTs initially interact with the MT lattice and subsequently attach to the plus ends of MTs; that is the lateral attachment is converted to end-on attachment (Figure 1, step 2; Figure 3B). Presumably, the Ndc80 complex is involved not only in the lateral attachment but also in the end-on attachment; consistent with this, an injection of an antibody against the Ndc80 CH domain changed the dynamics of KT–MT interactions in metaphase (DeLuca *et al.*, 2006), moreover the Ndc80 complex can couple a microsphere at the end of a dynamic MT in an *in vitro* reconstituted system (McIntosh *et al.*, 2008; Powers *et al.*, 2009).

Interface of the KT–MT attachment: the Dam1 complex and its functional equivalents

In yeast cells, the Dam1 complex also has a crucial role in the end-on KT–MT attachment. The Dam1 complex, also called DASH, is composed of 10 proteins and has been identified in budding yeast (reviewed in Westermann *et al.*, 2007; Nogales and Ramey, 2009) and fission yeast (Liu *et al.*, 2005; Sanchez-Perez *et al.*, 2005). The Dam1 complex is not a part of the KT during the lateral KT–MT attachment, and only upon end-on attachment, it is loaded on the KT (Tanaka *et al.*, 2007) (Figure 3B). The Dam1 complex has the ability to track the plus end of a shrinking MT (Westermann *et al.*, 2006; Tanaka *et al.*, 2007) and, once loaded on the KT, it mediates the end-on pulling of the KT by a shrinking MT in budding yeast (Tanaka *et al.*, 2007). A similar role is also suggested for the Dam1 complex in fission yeast (Franco *et al.*, 2007; Gachet *et al.*, 2008). During this process, the Dam1 complexes form oligomers and/or a ring structure encircling an MT (Figure 3B) (Miranda *et al.*, 2005; Westermann *et al.*, 2005; Wang *et al.*, 2007; Gestaut *et al.*, 2008; Grishchuk *et al.*, 2008).

During MT 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 MT depolymerization to a KT pulling force (Asbury *et al.*, 2006; Westermann *et al.*, 2006; Tanaka *et al.*, 2007).

The KT loading of the Dam1 complex, which occurs when the end-on KT–MT attachment is established (Tanaka *et al.*, 2007), is dependent on the Ndc80 complex (Janke *et al.*, 2002; Shang *et al.*, 2003; Wong *et al.*, 2007). Thus, the Ndc80 and the Dam1 complexes may work together to support the end-on KT–MT attachment (Figure 3B). Consistent with this notion, the Dam1 complex enhances the MT-binding affinity of the Ndc80 complex and facilitates its MT plus-end accumulation *in vitro* (Lampert *et al.*, 2010; Tien *et al.*, 2010). Furthermore, disrupting the association between the two complexes leads to a defect in the end-on KT–MT attachment, while the lateral attachment is still normal (our unpublished results).

The Dam1 complex has an important function in chromosome motion towards a spindle pole, soon after initial KT–MT interaction and before sister KT bi-orientation (Figure 1, step 2,

right; end-on pulling; Kitamura *et al.*, 2007; Tanaka *et al.*, 2007). Presumably, the complex has a similar role in anaphase A, where KTs move towards a spindle pole again by MT end-on pulling (Figure 1, step 5). In addition, the complex may also have a function in tension-coupled chromosome oscillation during metaphase (Franck *et al.*, 2007) (Figure 1, step 4). The important roles of the Dam1 complex in chromosome segregation is also supported by recent finding that Dam1 complexes tethered on a minichromosome lacking a centromere could facilitate its segregation during mitosis (Kiermaier *et al.*, 2009; Lacefield *et al.*, 2009).

Although the Dam1 complex has essential roles in KT association with the end of an MT in budding yeast, convincing orthologues of Dam1 components have not been identified in metazoan cells (Meraldi *et al.*, 2006). However, functional counterparts of the Dam1 complex may be present in metazoa, albeit with little homology in amino-acid sequences. Indeed, the Ska1 complex, consisting of Ska1, Ska2 and Rama1/Ska3, is required for proper chromosome segregation and shows similar functional properties to the Dam1 complex (Hanisch *et al.*, 2006; Daum *et al.*, 2009; Gaitanos *et al.*, 2009; Raaijmakers *et al.*, 2009; Welburn *et al.*, 2009). For example, as with the Dam1 complex, the Ska1 complexes can directly bind MTs, oligomerize into a ring-like structure and facilitate the processive motion of a microsphere along a depolymerizing MT *in vitro* (Welburn *et al.*, 2009). Moreover, similarly to the Dam1 complex, the KT loading of the Ska1 complex is dependent on Ndc80 (Hanisch *et al.*, 2006).

Furthermore, Cep57 and Bod1 are proposed to be alternative candidates for functional counterparts of the Dam1 complex in vertebrate cells (Emanuele and Stukenberg, 2007; Porter and Swedlow, 2007). From analogy to yeast (as discussed above), the Dam1 functional counterparts are expected to be involved in establishing the end-on KT–MT attachment; in *Caenorhabditis elegans*, this process seems to rely on interplay between spindly, dynein and the dynein-associated RZZ complex (Gassmann *et al.*, 2008).

So far, the Ndc80 complex, KNL1, the Dam1 complex and its functional equivalents are thought to interact with the MT outer surface. However, electron microscopy identified microfibrils in vertebrate cells, which extend from the KT to the inner surface of the MT tip in which protofilaments curl out (McIntosh *et al.*, 2008). Although the identity of such microfibrils is not yet known, they may also have an important function in tethering the KT at the dynamic MT plus end.

Mechanisms promoting sister KT bi-orientation: KT geometry and error correction

Following the KT transport towards a spindle pole (Figure 1, step 2), one sister KT becomes attached to MTs from one pole while the other sister to those from the other pole (sister KT bi-orientation; Figure 1, step 4). In order to achieve bi-orientation (also called amphitelic attachment; Figure 4), the aberrant KT–MT attachment, for example sister KTs attaching to MTs from the same spindle pole (syntelic attachment; Figure 4), must be either avoided or corrected before anaphase onset. Two kinds of mechanisms could promote this process: the KT geometry (for avoidance) and tension-dependent error correction.

Figure 4 Modes of kinetochore–microtubule interactions. Monotelic attachment: one of the sister KTs attaches to MTs, whereas the other does not attach to any MTs. Syntelic attachment: both sister KTs attach to MTs extending from one spindle pole. As a result of monotelic or syntelic attachment, sister KTs ‘mono-orient’; that is, they are connected to only one spindle pole directly or indirectly. Amphitelic attachment: one sister kinetochore becomes attached to microtubules from one pole while the other sister to those from the other pole. As a result of amphitelic attachment, sister KTs ‘bi-orient’; that is they are connected to the opposite spindle poles. Merotelic attachment: one sister KT simultaneously attaches to MTs extending from both spindle poles. The figure is adapted from a figure in Tanaka *et al* (2005b).

The KT geometry relies on a back-to-back position of sister KTs. When one KT attaches to MTs from one spindle pole (monotelic attachment; Figure 4), the constraint in geometry makes the other face the opposite direction, allowing association with MTs only from the opposite pole (Ostergren, 1951). For sister KT geometry, cohesion between sister centromeres is thought to have important functions (Tanaka *et al*, 1999). More specifically, it was suggested that reduced cohesion at the core centromere, relative to para-centromere regions, facilitates sister KT geometry for bi-orientation in fission yeast (Sakuno *et al*, 2009). In budding yeast, the core centromeres are looped out from para-centromeres (Yeh *et al*, 2008; Anderson *et al*, 2009), which may contribute to this geometry. Thus, an aberrant KT–MT attachment could be avoided by KT geometry.

However, once aberrant attachment is made, the geometry mechanism can no longer correct it. In this circumstance, error correction is important in removal of aberrant KT–MT attachment (Figure 1, step 3). Such error correction was first discovered in meiosis I, where two homologous KTs must bi-orient on the spindle. Here, KTs and spindle poles repeatedly become connected (by MTs) and disconnected until bi-orientation is established (Nicklas, 1997). Using micro-needle manipulation in grasshopper spermatocytes, Nicklas and Koch (1969) showed that tension applied on chromosomes leads to stabilization of KT–spindle-pole connections by MTs.

The importance of tension for KT bi-orientation was also tested in mitosis; an unreplicated circular minichromosome with two centromeres was engineered and its behaviour was observed in budding yeast (Dewar *et al*, 2004). On this minichromosome, the two centromeres would lack back-to-back geometry, but tension should be generated across them by inter-centromere chromatin, when two centromeres bi-orient on the spindle. Such a minichromosome showed efficient bi-orientation, suggesting that tension across its two KTs is sufficient to promote bi-orientation (Dewar *et al*, 2004).

The corollary is that, on normal sister KTs, stable KT–MT attachment is established reliant on tension across sister KTs (Figure 1, steps 3 and 4). If KT–MT attachment does not generate tension as happens during syntelic attachment, such attachment would be removed to allow formation of a fresh KT–MT attachment. When bi-orientation is established, tension is generated across sister KTs, leading to stable KT–MT attachment, thus completing the error correction (Nicklas and Koch, 1969; Nicklas, 1997; Dewar *et al*, 2004).

In this process of turnover of the KT–MT attachment, cohesion between sister centromeres is necessary to generate tension when bi-orientation is established. Thus, cohesion between sister centromeres is important not only for KT geometry (see above), but also for the error correction mechanism. Cohesin proteins are required for sister chromatid cohesion (Nasmyth, 2002) and they are indeed necessary to ensure sister KT bi-orientation (Tanaka *et al*, 2000; Sonoda *et al*, 2001; Dewar *et al*, 2004; Vagnarelli *et al*, 2004; Eckert *et al*, 2007).

In vertebrate cells, mono-oriented KTs (with monotelic or syntelic attachment; Figure 4) can move from the vicinity of a spindle pole to the middle of the spindle, along MTs that attach to other already bi-oriented KTs (Kapoor *et al*, 2006). This motion should enhance the chance for such KTs to interact with MTs from the opposite pole, thus providing an additional mechanism to facilitate turnover of KT–MT attachments, leading to bi-orientation.

Although the tension-dependent error correction seems to suffice for efficient establishment bi-orientation in mitosis (see above), KT geometry may also facilitate bi-orientation as a redundant mechanism (Indjeian and Murray, 2007; Sakuno *et al*, 2009). Thus, sister KT geometry is generally thought to assist bi-orientation. However, KTs tend to maintain their geometry in mammalian cells and, following syntelic attachment, sister KTs may remain juxtaposed even after aberrant KT–MT attachment is removed (Loncarek *et al*, 2007). In this circumstance, KT geometry has to be overridden by the error correction for bi-orientation to become established.

In budding yeast, only one MT is thought to attach to a single KT in metaphase (Winey *et al.*, 1995). In contrast, multiple MTs attach to a single KT in fission yeast and metazoan cells (McDonald *et al.*, 1992; Ding *et al.*, 1993). In this situation, errors could occur in such a way that a single KT becomes attached to MTs from opposite spindle poles (merotelic attachment; Figure 4). How do cells avoid or correct this type of error? Merotelic attachments are probably discouraged by the KT geometry. For example, a candidate KT clamp ensuring this geometry has been suggested in fission yeast (Gegan *et al.*, 2007).

However, KT geometry may not be sufficient to avoid merotelic attachment. For example, the presence of an extra spindle pole often causes merotelic attachments, following the fusion of two spindle poles (Ganem *et al.*, 2009); this would be difficult to avoid by KT geometry. In fact, if merotelic attachments are formed, they can still be corrected before anaphase onset (see the next section; Cimini *et al.*, 2003; Cimini *et al.*, 2006). Even if merotelic attachments persist until anaphase, the imbalance between MT forces applied on sister KTs could lead to their proper segregation, albeit with some delay (Cimini *et al.*, 2004).

Regulators for the error correction: Aurora B/Ipl1 kinase

What factors are involved in the error correction to facilitate sister KT bi-orientation? A series of evidence suggests that Aurora B (called Ipl1 in budding yeast), an evolutionarily conserved serine/threonine protein kinase, has a crucial role in this process.

When Ipl1 is defective in yeast cells, KTs are able to interact with MTs; however, sister KT bi-orientation is defective in the majority of cells (Biggins and Murray, 2001; He *et al.*, 2001; Tanaka *et al.*, 2002). In an *ipl1* mutant, an unreplicated circular minichromosome with two centromeres (see above) often failed to bi-orient, suggesting that Ipl1 could facilitate bi-orientation, independently of KT geometry (Dewar *et al.*, 2004). Moreover, in this mutant, the KT failed to change its MT association in the absence of tension, in contrast to wild-type cells (Tanaka *et al.*, 2002; Dewar *et al.*, 2004). It was, therefore, suggested that Ipl1 kinase promotes turnover of the KT–MT interaction to eliminate KT–MT interactions that do not generate tension across sister KTs (Tanaka *et al.*, 2002) (Figure 5). Aurora B has a similar role in mammalian cells, as they accumulated syntelic KT–MT attachments when this kinase was defective (Hauf *et al.*, 2003; Lampson *et al.*, 2004).

Aurora B/Ipl1 kinase localizes at centromeres in prometaphase and metaphase and it can phosphorylate KT components, which is thought to weaken the KT–MT attachment and facilitate its turnover (Figure 5, left). Crucial substrates of this kinase include the Dam1-complex components in budding yeast (Cheeseman *et al.*, 2002; Zhang *et al.*, 2005), and Ndc80 (Cheeseman *et al.*, 2006; DeLuca *et al.*, 2006; Ciferri *et al.*, 2008; Akiyoshi *et al.*, 2009a) and KNL1 (Liu *et al.*, 2010; Welburn *et al.*, 2010) in yeast and mammalian cells. Phosphorylation of Dam1 is clustered at its C-terminus and reduces interaction with the Ndc80 complex (see the section above) (Cheeseman *et al.*, 2002; Shang *et al.*, 2003; Lampert *et al.*, 2010; Tien *et al.*, 2010). On the other hand, phosphorylation of Ndc80 is clustered at its N-terminal basic region

Figure 5 How Aurora B/Ipl1 kinase promotes the error correction of kinetochore–microtubule attachment. The Aurora B/Ipl1 kinase facilitates sister KT bi-orientation by promoting the turnover of KT–MT attachment in a tension-dependent manner; this process is thought to take place as follows (Tanaka *et al.*, 2002; Dewar *et al.*, 2004; Liu *et al.*, 2009). While syntelic attachment does not generate tension across sister KTs (left), the Aurora B/Ipl1 kinase phosphorylates KT components such as Ndc80 and Dam1 (see references in text), which promotes turnover of the KT–MT attachment. When bi-orientation is established as a result of this turnover (right), tension is applied across sister KTs, causing delocalization of the KT substrates from Aurora B/Ipl1, which leads to their de-phosphorylation. This in turn makes the KT–MT attachment more stable and stops the turnover of this attachment. Thus, sister KT bi-orientation is stably maintained and the error correction is completed for the KT–MT attachment.

and reduces the affinity of the Ndc80 complex for MTs (Cheeseman *et al.*, 2006; Ciferri *et al.*, 2008) and inter-complex association (Ciferri *et al.*, 2008; Alushin *et al.*, 2010). Moreover, *dam1*, *ndc80* and *kn1* mutants mimicking constitutive de-phosphorylation show defects in bi-orientation (Cheeseman *et al.*, 2002; DeLuca *et al.*, 2006; Welburn *et al.*, 2010), suggesting that their phosphorylation by Aurora B/Ipl1 is crucial for bi-orientation.

In metazoan cells, multiple MTs attach to a single KT, where Aurora B may have a more complex function 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 section above; Figure 4). In vertebrate cells, the MT-depolymerizing activity of MCAK is regulated by Aurora B, which could be important in removing syntelic and merotelic attachments (Ohi *et al.*, 2003; Andrews *et al.*, 2004; Lan *et al.*, 2004; Ohi *et al.*, 2004; Knowlton *et al.*, 2006; Zhang *et al.*, 2007).

Meanwhile, once bi-orientation is established and tension is applied on KTs, cells must stop the turnover of the KT–MT attachment (Figure 5, right) (Tanaka *et al.*, 2002; Pearson *et al.*, 2004); otherwise, bi-orientation would not be stably maintained. It was demonstrated that Aurora B/Ipl1 substrates Dam1 and KNL1, whose phosphorylation is important for bi-orientation, are de-phosphorylated when sister KTs bi-orient and tension is applied across sister KTs (Keating *et al.*, 2009; Welburn *et al.*, 2010).

How does this de-phosphorylation occur when tension is applied on sister KTs? Evidence suggests that when tension is applied, Aurora B/Ipl1 substrates at the KTs are pulled out due to spindle MT forces and, therefore, delocalize from the Aurora B/Ipl1 kinase that is enriched at inner centromere regions (Figure 5, right). This was first suggested in budding yeast (Tanaka *et al.*, 2002; Shimogawa *et al.*, 2009) and subsequently demonstrated using a FRET sensor in mammalian cells (Liu *et al.*, 2009; Welburn *et al.*, 2010). This notion is consistent with the reports that intra-KT stretching becomes prominent upon sister KT bi-orientation (Maresca and Salmon, 2009; Uchida *et al.*, 2009), which will facilitate delocalization of outer KTs (including Aurora B/Ipl1 substrates) from this kinase.

As an alternative mechanism, it is proposed that the kinase activity of Aurora B/Ipl1 might be regulated by tension. For example, Bir1 and Sli15 (Survivin and INCENP in metazoa), binding partners of Ipl1 (reviewed in Ruchaud *et al.*, 2007; Carmena *et al.*, 2009), are proposed to act as a tension sensor (Sandall *et al.*, 2006). Another model proposes that MTs, aberrantly associated with KTs, may enhance the activity of Aurora B kinase (Rosasco-Nitcher *et al.*, 2008).

Upon anaphase onset, Aurora B/Ipl1 kinases relocate from KTs to the spindle, thus they are also known as ‘chromosome passenger complex (CPC)’, together with binding partners Survivin/Bir1 and INCENP/Sli15 (reviewed in Ruchaud *et al.*, 2007). This relocation is regulated by Cdc14 phosphatase (Pereira and Schiebel, 2003; Mirchenko and Uhlmann, 2010), cyclin B degradation (Parry *et al.*, 2003; Oliveira *et al.*, 2010) and a kinesin-6 member Mklp2 (Gruneberg *et al.*, 2004; Hummer and Mayer, 2009; Vazquez-Novelle and Petronczki, 2010), as shown in yeast, fly and mammalian cells, respectively. The Aurora B/Ipl1 relocation from the KT is presumably important to stop turnover of KT–MT attachment during anaphase, in which tension on the KT is considerably reduced. In fact, when this relocation of Aurora B/Ipl1 is inhibited, KTs show continuous back-and-forth motion on the anaphase spindle (Parry *et al.*, 2003; Oliveira *et al.*, 2010) and the SAC is re-engaged (Mirchenko and Uhlmann, 2010; Oliveira *et al.*, 2010); these events probably reflect the resumed turnover of the KT–MT attachment in anaphase.

More regulators for the error correction: Bub1, Sgo, Haspin, PP1 and Mps1

Several regulators are involved in targeting the CPC (including Aurora B/Ipl1) at centromeres in early mitosis, thus ensuring the error correction in KT–MT attachment. Recent studies have revealed that two pathways ensure this process. In the first pathway, Bub1 kinase phosphorylates histone H2A at centromere regions (Kawashima *et al.*, 2010), which recruits Sgo proteins (Sgo1 in budding yeast and mammals; Sgo2 in fission yeast) that in turn bind Bir1 (fission yeast) and another CPC component Borealin (mammals) when they are phosphorylated by cyclinB/CDK (Tsukahara *et al.*, 2010). In the second pathway, Haspin kinases phosphorylate histone H3 at threonine 3 (Dai *et al.*, 2005), which binds Survivin/Bir1 (Kelly *et al.*, 2010; Wang *et al.*, 2010; Yamagishi *et al.*, 2010).

While the two pathways work partially redundantly, Aurora B is enriched at inner centromere regions where both pathways are active (Yamagishi *et al.*, 2010). Indeed, sister KT bi-orientation and resultant chromosome congression

become partially defective in yeast and mammalian cells with mutation or depletion of Bub1 (Asakawa *et al.*, 2005; Meraldi and Sorger, 2005; Windecker *et al.*, 2009), Sgo (Fernius and Hardwick, 2007; Kawashima *et al.*, 2007; Vanoosthuyse *et al.*, 2007; Kiburz *et al.*, 2008) or Haspin (Dai *et al.*, 2005). Notably, defects in both pathways cause more extensive chromosome mis-segregation (Yamagishi *et al.*, 2010).

Meanwhile, PP1 (called Glc7 in budding yeast) is a phosphatase, which is thought to implement de-phosphorylation of Aurora B/Ipl1 substrates (reviewed in De Wulf *et al.*, 2009). Consistent with this notion, Glc7 mutants show a defect in the KT–MT interaction (Bloecher and Tatchell, 1999; Sassoon *et al.*, 1999). It was recently suggested that Fin1 and KNL1 are associated with Glc7 and PP1, respectively, to facilitate their recruitment to the KT (Akiyoshi *et al.*, 2009b; Liu *et al.*, 2010). In particular, the KNL1–PP1 association is enhanced when Aurora B-dependent KNL1 phosphorylation is reduced (Liu *et al.*, 2010), constituting a possible positive feedback loop for rapid de-phosphorylation of Aurora B substrates at the KT when tension is applied across sister KTs upon bi-orientation.

Mps1 is an evolutionarily conserved protein kinase, which is required for the SAC and, in some organisms, for duplication of MTOCs of the mitotic spindle (reviewed in Winey and Huneycutt, 2002). On the other hand, independent of these functions, Mps1 has an important role in chromosome segregation (Jones *et al.*, 2005), especially in sister KT bi-orientation (Maure *et al.*, 2007; Araki *et al.*, 2010). Similarly to Aurora B/Ipl1, Mps1 promotes turnover of any KT–MT attachment that does not generate tension in budding yeast (Maure *et al.*, 2007). Similarly, in mammalian cells, Mps1 is required for the error correction of the KT–MT attachment (Jelluma *et al.*, 2008). It is currently a matter of debate whether Mps1 regulates the Aurora B kinase (Jelluma *et al.*, 2008; Sliedrecht *et al.*, 2010), or *vice versa* (Hewitt *et al.*, 2010; Santaguida *et al.*, 2010), or they work in parallel pathways (Maure *et al.*, 2007; Maciejowski *et al.*, 2010) to promote sister KT bi-orientation.

Error correction and SAC: intertwined relationship

The spindle-assembly checkpoint (SAC) is a surveillance mechanism that delays anaphase onset if sister KTs fail to bi-orient, or if any KTs fail to attach to MTs (reviewed in Musacchio and Salmon, 2007; Burke and Stukenberg, 2008; Khodjakov and Rieder, 2009). The SAC and error correction are often argued in the same context, but they are distinct mechanisms having different effects. While the error correction selectively promotes bi-orientation, the SAC does not promote bi-orientation by itself, but can buy the time for the error correction to establish bi-orientation. Although the error correction is an essential mechanism for cell growth in any eukaryote so far studied, the SAC is not essential for cell proliferation as long as cells have sufficient time to establish bi-orientation, as is the case in budding and fission yeast (reviewed in Lew and Burke, 2003) and *Drosophila* (Biffin *et al.*, 2007).

Nonetheless, the SAC often remains unsatisfied in situations where the error correction must operate to promote bi-orientation. For example, in both the SAC and error correction, a lack of tension is somehow ‘sensed’ on KTs, engaging cells to take required actions. Therefore, it may not

be surprising that common regulators are used at the KT both to signal the SAC and to facilitate the error correction. Aurora B/Ipl1, Mps1 (reviewed in Nezi and Musacchio, 2009), Sgo (Indjeian *et al*, 2005) and Bub1 (Asakawa *et al*, 2005; Windecker *et al*, 2009) are the examples of such common regulators. Given that SAC remains unsatisfied when KTs lack MT attachment, the involvement of Aurora B/Ipl1 and Mps1 (and Sgo and Bub1 as they recruit Aurora B/Ipl1 at the KT; see above) in both pathways may be explained as follows: these kinases may transiently create the MT-free KTs by facilitating the turnover of the KT–MT attachment, thus keeping the SAC unsatisfied (Tanaka *et al*, 2002; Ditchfield *et al*, 2003; Pinsky *et al*, 2006).

However, the actual relationship between the SAC and error correction might be more complex. For example, the following results cannot be explained by the above ‘simple’ explanation: (1) Mps1 is required for the SAC in nocodazole-treated cells, where KTs lack MT attachment (Winey and Huneycutt, 2002), (2) Aurora B is also required for the SAC in the same circumstance, at least in some organisms (Kallio *et al*, 2002; Petersen and Hagan, 2003), (3) Mad3 phosphorylation by Ipl1 is required for the SAC, but not for the error correction, in the absence of tension (King *et al*, 2007) and (4) phosphorylation of Ndc80 N-terminus by Mps1 is required for the SAC, but not for establishing sister KT bi-orientation (Kemmler *et al*, 2009). Thus, phosphorylation of the KT components and SAC components by Aurora B/Ipl1 and Mps1 kinases may signal the SAC, in part, independently of the turnover of KT–MT attachment.

Conclusions and perspectives

Establishing sister KT bi-orientation is a pivotal process ensuring equal segregation of the genetic information into daughter cells upon cell division. In particular, the tension-dependent error correction is a crucial mechanism to secure sister KT bi-orientation. Sister KT bi-orientation and the error correction are not only fundamental for normal cell growth,

but might also be important to prevent generation of cancers. Indeed, a recent report suggested that KT–MT attachment is more stable in cancer cells than in normal cells (Bakhoun *et al*, 2009), arguing that persistence of aberrant KT–MT attachment underlies the chromosome instability found in cancer cells. Thus, a defect in the error correction may have a causative function in generation of some type of cancers.

To understand the steps towards sister KT bi-orientation, many pertinent questions remain to be answered. For example, how do the Ndc80 complex, Dam1 complex (or a functional equivalent) and KNL1 cooperate to convert the lateral KT–MT attachment to the end-on attachment? What is the relative contribution of the error correction and KT geometry towards sister KT bi-orientation? What processes do cells undergo during turnover of the KT–MT attachment leading to the error correction; for example how is the old attachment removed and the new one established? How does Aurora B/Ipl1 promote turnover of the KT–MT attachment through phosphorylation of KT substrates? How does Mps1 contribute to the error correction; if Aurora B/Ipl1 is not the only target of Mps1, what are crucial substrates of Mps1 for the error correction? The combined efforts in biochemistry, structural analyses, biophysics, genetics, cell biology and *in silico* study will advance research in this field.

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Conflict of interest

The author declares that he has no conflict of interest.

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