Kinetochore–microtubule interactions: the means to the end
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Kinetochore is a proteinaceous complex containing dozens of components; they are assembled at centromeric DNA regions and provide the major microtubule attachment site on chromosomes during cell division. Recent studies have defined the kinetochore components comprising the direct interface with spindle microtubules, primarily through structural and functional analysis of the Ndc80 and Dam1 complexes. These studies have facilitated our understanding of how kinetochores remain attached to the end of dynamic microtubules and how proper orientation of a kinetochore–microtubule attachment is promoted on the mitotic spindle. In this article, we review these recent studies and summarize their key findings.

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Introduction
To maintain their genetic integrity, eukaryotic cells must segregate their replicated chromosomes properly during mitosis. Chromosome segregation is dependent on interactions between microtubules and the kinetochore, a large proteinaceous complex assembled on the centromere regions of chromosomes during mitotic entry (reviewed in references [1–3]). For high-fidelity chromosome segregation, kinetochores must capture spindle microtubules and connect the sister chromatids of all chromosomes to opposite spindle poles before anaphase onset. Proper kinetochore capture of spindle microtubules is achieved in a stepwise manner (reviewed in references [4,5]; Figure 1). Kinetochore initially attach to the surface of a single microtubule that extends from either spindle pole [6–8] (Figure 1, step 1). Once bound, kinetochores are transported poleward along microtubules (Figure 1, step 2). During or after this transport, both sister kinetochores interact with microtubules.

If kinetochores are wrongly attached, as occurs during syntelic attachment (where both sister kinetochores connect to microtubules from the same spindle pole), the kinetochore–spindle pole connections must be re-oriented (Figure 1, step 3) to convert to proper bi-orientation (i.e. attachments of sister kinetochores to microtubules extending from the opposite spindle poles; step 4), before anaphase onset is triggered (reviewed in references [5,9]).

During the course of achieving bi-orientation, kinetochores exhibit two distinct types of associations with spindle microtubules. Initially kinetochores interact laterally with the microtubules lattice. Subsequently they are tethered at the microtubule plus end and exhibit motility directly coupled to microtubule polymerization and depolymerization (end-coupled attachment). A major question in the field has been to define the molecular mechanisms operating during these two types of attachments.

After bi-orientation, kinetochores and chromosome arms are aligned on the equatorial plate of the mitotic spindle (metaphase plate); this process is called congression [10]. Once all kinetochores bi-orient and congress on the spindle, cohesion between sister chromatids is removed (reviewed in reference [11]). Then each sister kinetochore, attached to the plus end of microtubules, is pulled towards the opposite spindle poles during anaphase (Figure 1, step 5). Sister separation during anaphase A, when chromosome-pole distance decreases, is coupled to microtubule depolymerization that occurs at the kinetochore (microtubule plus end) and, in the case of metazoan cells, also near centrosomes (microtubule minus end; reviewed in references [12,13]).

In this article, we review recent papers (over the past 2 years), focusing on the following aspects of kinetochore–microtubule interactions: (1) The kinetochore is a large complex composed of dozens of proteins (reviewed in references [3,14,15]); which of these proteins form the direct interface with spindle microtubules during lateral and end-coupled attachments? (2) How do kinetochores remain attached to the ends of depolymerizing microtubules? (3) While microtubule depolymerization is an important driving force for kinetochore movement, how do microtubule motor proteins contribute to kinetochore motility, especially in the initial steps of kinetochore–microtubule interactions? (4) How is sister kinetochore bi-orientation promoted on the mitotic spindle by re-orientation of kinetochore–spindle pole connections?
Overview of kinetochore–microtubule interactions. The figure depicts kinetochore–microtubule interactions during prometaphase (steps 1–3), metaphase (step 4) and anaphase A (step 5).

1) Kinetochore capture by the lateral surface of a microtubule

2) Microtubule-dependent kinetochore transport towards a spindle pole

3) Interaction of sister kinetochores with microtubules from the same or opposite spindle poles

4) Sister kinetochore bi-orientation (tension applied)

5) Sister chromatid separation

(1) Kinetochores are initially captured by the lateral surface of single microtubules that extend from one of the spindle poles [6–8]. The initial kinetochore encounter with microtubules happens quickly following nuclear envelope breakdown (metazoan cells) [6,7] or once kinetochore assembly is complete (budding yeast: note that spindle poles of this organism have not yet separated in steps 1–2) [53].

(2) Once captured, kinetochores are transported along the lateral surface of single microtubules towards the spindle pole (sliding) [6–8]. Subsequently, at least in budding yeast, kinetochores are tethered at the end of the single microtubules and transported further as the microtubules shrink (end-coupled pulling) [40*].

(3) As kinetochores approach spindle poles, both sister kinetochores attach to microtubules. If both kinetochores attach to microtubules from the same spindle pole, kinetochore–spindle pole connections by microtubules are re-oriented until proper bi-orientation is established [5,9].
When addressing these questions, we will emphasize work on two protein complexes that have been the subject of intense recent study: the Ndc80 complex and the Dam1 complex. We will not discuss kinetochore composition and assembly [3,14,15], the spindle-assembly checkpoint [16], dynamics of spindle and kinetochore microtubules [17–19], or kinetochore–microtubule interactions in meiosis [20,21]. These topics have been recently reviewed in the indicated references.

The Ndc80 complex: a key component of the kinetochore–microtubule interface

Recent studies have revealed that the Ndc80 complex, an outer kinetochore component conserved from yeast to humans (reviewed in references [3,22]), comprises a centrally important constituent of the kinetochore that directly interacts with microtubules. Depletion or inactivation of the Ndc80 complex causes the most severe chromosome segregation defect observed following inhibition of an outer kinetochore component. The complex is composed of four proteins: Ndc80 (also called Hec1 in mammals), Nuf2, Spc24 and Spc25 (Figure 2a, top). Ndc80-Nuf2 and Spc24-Spc25 form heterodimers with a globular domain at one side and a coiled-coil shaft at the other [23–25]. The two heterodimers are held together by interaction of their coiled-coil shafts, making a heterotetrameric arrangement of approximate 80–100 amino acids in length that extends out of the CH domain of Ndc80 is crucial for microtubule association. Following deletion of this region, the affinity of the Ndc80/Nuf2 dimer for microtubules is precipitously decreased [31**]. The association of the Ndc80 subcomplex with microtubules also exhibits cooperativity [30**], which may be important in the context of forming multivalent interactions with single microtubules. The CH domain of EB1 interacts with microtubules preferentially along the microtubule seam [34*] (where the two-dimensional protofilament sheet of a microtubule is finally closed [35]), whereas the Ndc80 complex appears to bind along the entire microtubule lattice [30**,31**]; the reason for this difference remains to be characterized.

Most significantly, biochemical analysis and electron microscopy of the reconstituted Ndc80 complex have shown that the Ndc80-Nuf2 subunits (in particular, their globular domains) directly interact with the microtubule lattice, albeit with a low affinity [30**,31**] (Figure 2b). The crystal structure of the globular domain of the Ndc80 subunit has revealed that it is folded into a calponin-homology (CH) domain [31**]. Intriguingly, the microtubule-binding region of the plus-end-associated protein EB1 also forms a CH domain [32]; this similarity was not anticipated from the primary sequence and was revealed by the structure. CH domains were first discovered and characterized in actin-binding proteins such as α-actinin [33]. The presence of CH domains in EB1 and in the globular domain of Ndc80 indicates that this structural motif is also utilized in microtubule-binding proteins and suggests an ancient evolutionary origin for this fold; whether a similar domain protein interacts with prokaryotic actin and tubulin-like polymers will be interesting to investigate in future work.

For the Ndc80/Nuf2 dimer, an unstructured basic region of approximately 80–100 amino acid length that extends out of the CH domain of Ndc80 is crucial for microtubule association. Following deletion of this region, the affinity of the Ndc80/Nuf2 dimer for microtubules is precipitously decreased [31**]. The association of the Ndc80 complex with microtubules also exhibits cooperativity [30**], which may be important in the context of forming multivalent associations with single microtubules. The CH domain of EB1 interacts with microtubules preferentially along the microtubule seam [34*] (where the two-dimensional protofilament sheet of a microtubule is finally closed [35]), whereas the Ndc80 complex appears to bind along the entire microtubule lattice [30**,31**]; the reason for this difference remains to be characterized.

Consistent with its lattice binding activity, the Ndc80 complex is required for kinetochore association with the microtubule lateral surface in vivo in budding yeast [8]. There is also evidence that the Ndc80 complex plays a crucial role in end-coupled kinetochore attachments that predominate during bi-orientation, congression and segregation. Of particular note is a recent study in mammalian cells where injection of an antibody to an epitope in the globular domain of the Ndc80 subunit resulted in the opposite phenotype to Ndc80-complex inhibition [36*]; instead of loss of kinetochore–microtubule interactions, the attachment between the kinetochore and the spindle was hyper-stabilized, resulting in reduced turnover of bound microtubules and increased stretching of centromeric chromatin between sister kinetochores.

The KMN network: a conserved core protein group of the outer kinetochore

The Ndc80 complex is directly associated with KNL1 (Spc105/Spc7 in budding and fission yeasts, respectively) and the 4-subunit Mis12 complex, forming a larger

(Figure 1 Legend Continued)

(4) Cessation of re-orientation is dependent on the tension that is generated by microtubules upon establishment of bi-orientation [5,9]. The number of microtubules whose plus ends attach to a single kinetochore increases when tension is applied in metazoan cells [107], while only a single microtubule is thought to attach to a each kinetochore in budding yeast [62] (the latter case is shown here for simplicity).

(5) Once all kinetochores bi-orient on the spindle, cohesion between sister chromatids is removed, causing sister chromatid segregation to opposite spindle poles during anaphase A [11]. Kinetochores are end-coupled and pulled poleward as the microtubules depolymerize [12,13].
The Ndc80 complex and the KMN network.

(a) Schematic of the 4-subunit Ndc80 complex indicating the constituent parts and defined domains. Panels on the right show the rod-like structure of the complex visualized by electron microscopy of individual rotary-shadowed recombinant complexes (scale bar 100 nm; reprinted from reference [23], © 2005 National Academy of Sciences, USA). The ribbon diagrams below show the calponin-homology domain of the Ndc80 subunit (residues 81–196 of human Ndc80, also known as Hec1) and comparison with the EB1 CH domain (reprinted from reference [31]).
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In vitro experiments with purified tubulin have indicated that protofilament curling at the microtubule plus end can produce forces that are more than sufficient to move chromosomes towards a spindle pole [47]. A mathematical model has predicted that a microtubule-encircling ring, if present, would facilitate this process [48]. Thus, the Dam1-complex ring is an ideal machine to link chromosome movement to polymerization dynamics at end-coupled kinetochore attachments. In vitro biophysical studies have revealed that the recombinant Dam1 complex can effectively harness the intrinsic microtubule depolymerization-generated force and is also able to promote polymerization when placed under tension, both attributes of end-coupled kinetochore attachments [49*,50*]. The process of kinetochore poleward movement coupled to depolymerization was recently visualized in vivo in budding and fission yeast cells [40**,51,52,53*] (end-coupled pulling; Figure 1, step 2, right; Figure 3c). End-coupled poleward movement of kinetochores was dependent on the Dam1 complex in vivo [40**,52], as suggested by the in vitro studies. Presumably, this Dam1 function is also important during anaphase A, when end-coupled kinetochores are pulled polewards concomitant with depolymerization of plus ends at the kinetochore [12,13,54] (Figure 1, step 5). Thus, the Dam1 complex is the perfect molecular device that, together with the Ndc80 complex, would help generate the special properties of end-coupled attachments that are central to chromosome segregation.

Do metazoans have functional counterparts of the Dam1 complex?

The principles of Dam1-complex function are likely to be important for kinetochore–microtubule interactions in all eukaryotic cells. However, convincing orthologues of the Dam1 complex have not been identified in metazoans either by sequence searches or using genome-wide functional analysis [55,56]. This discrepancy might be explained by either of the following two scenarios: Dam1-complex orthologues, which form a ring encircling a microtubule, might be present in metazoans, but their peptide sequence might be too divergent to be identified. This scenario may be rebutted by a lack of a detectable ring structure at a kinetochore in electron tomography [38*]; however, such rings may not generate sufficient contrast for visualization by electron microscopy of cells and no rings have been observed at budding-yeast kinetochores [57,58].

**Figure 2 Legend Continued**

(b) Microtubule binding of the Ndc80 complex. The Ndc80 complex binds to the microtubule lattice with a fixed orientation forming ‘barbs’ that extend away from the lattice. The microtubule-binding activity is located in the globular region of the Ndc80/NuF2 dimer and is severely reduced by removal of the N-terminal extension on Ndc80. The ‘barbs’ have a uniform polarity and binding angle indicating a specific binding site on the lattice (scale bar 200 nm; reprinted from reference [30**]).

c) Schematic of the KNL-1/Mis12 complex/Ndc80 complex (KMN) complex network. Direct association of the Ndc80 complex with these other two kinetochore constituents is conserved in fungi, nematodes, insects and vertebrates [3]. The Spc24/25 dimer is required for association with KNL-1 and the Mis12 complex. KNL-1 also directly binds to microtubules. Aurora B negatively regulates the microtubule-binding activity of the Ndc80 complex by phosphorylating the basic N-terminal extension of the Ndc80 subunit [30**].
where the Dam1 complex is clearly present in numbers sufficient to form a ring and important for end-coupled attachments [29,40]. Second, the ring structure might be dispensable for kinetochore–microtubule interaction and therefore absent in metazoa; meanwhile other molecules may compensate for this function. Relevant to this scenario is the finding that Dam1-complex components are not essential for cell viability in fission yeast [59,60], in contrast to budding yeast; this difference may reflect the fact that a single versus multiple microtubules attach to a single kinetochore during metaphase of budding and fission yeasts, respectively [61,62].

Four possible functional counterparts (orthologues or compensatory factors) of the Dam1 complex have been suggested at vertebrate kinetochores (all are outer kinetochore components, except for kinesin-13). Kinesin-13 (MCAK, etc.), which facilitates microtubule depolymerization [63], is a substrate of Aurora B kinase [64–68,69], similar to Dam1 (see below) and can form rings/spirals encircling a microtubule at least in vitro [70,71,72]. However, the ring/spiral structures formed by MCAK are distinct from those formed by the Dam1 complex and structural comparisons suggest that they would not be as effective as the Dam1 complex in coupling to polymerization dynamics [73]. Ska1/2 are proteins identified in vertebrates that require kinetochore–microtubule attachment for their recruitment to kinetochores and, once recruited, somehow modulate this attachment similarly to the Dam1 complex [39]. Cep57 also contributes to kinetochore–microtubule attachment and exhibits weak sequence similarity to Dam1 [69]. Bod1 is necessary for ensuring sister kinetochore bi-orientation similarly to the Dam1 complex [41]. Further studies on these interesting proteins are needed to understand their mechanistic contributions to chromosome segregation as well as to establish their relationship to the fungal Dam1 complex.
**Motor proteins associated with kinetochores**

Several motor proteins are associated with kinetochores and play important roles in microtubule-dependent kinetochore motion (reviewed in references [2,76]). In the initial stages of kinetochore–microtubule interactions, kinetochores associate with the microtubule lateral surface and are transported towards a spindle pole [6–8] (sliding; Figure 1, step 2, left). Kinetochore sliding is often converted to end-coupled attachment that exerts a poleward pulling force on the kinetochore (see the above section; Figure 1, step 2), but the opposite conversion is rare [40]. Kinetochore sliding occurs rapidly (10–50 μm/min) towards a spindle pole in vertebrate cells [77,78], but much more slowly (1–1.5 μm/min) and accompanied by transient pausing in budding yeast [8,40]. Such difference is attributed to the use of different microtubule minus-end directed motors associated with kinetochores; dynein, a processive motor, is used in metazoans [78,79], whereas Kar3, a kinesin-14 family member and non-processive motor (i.e. the motor is released from microtubules after each ATPase cycle [80]), is used in budding yeast [8,40]. Dynein localizes only outside of nuclei in yeast; presumably, upon the evolution of open mitosis [81], eukaryotic cells acquired the ability to use dynein in nuclear functions.

After kinetochores are transported to the vicinity of a spindle pole in prometaphase, they move towards the spindle equator to form the metaphase plate (congression) (Figure 1, step 2, left). Kinetochore sliding is often converted to end-coupled attachment that exerts a poleward pulling force on the kinetochore (see the above section; Figure 1, step 2), but the opposite conversion is rare [40]. Kinetochore sliding occurs rapidly (10–50 μm/min) towards a spindle pole in vertebrate cells [77,78], but much more slowly (1–1.5 μm/min) and accompanied by transient pausing in budding yeast [8,40]. Such difference is attributed to the use of different microtubule minus-end directed motors associated with kinetochores; dynein, a processive motor, is used in metazoans [78,79], whereas Kar3, a kinesin-14 family member and non-processive motor (i.e. the motor is released from microtubules after each ATPase cycle [80]), is used in budding yeast [8,40]. Dynein localizes only outside of nuclei in yeast; presumably, upon the evolution of open mitosis [81], eukaryotic cells acquired the ability to use dynein in nuclear functions.

Mechanisms ensuring sister kinetochore bi-orientation

In addition to proteins necessary for the kinetochore–microtubule attachment, what factors are required to ensure sister kinetochore bi-orientation before anaphase onset? Aurora B (Ipl1 in budding yeast) kinase is a key regulator for bi-orientation [84–87], and it was suggested that this kinase promotes turnover of kinetochore–spindle pole connections and eliminates those that do not generate tension between sister kinetochores [87–90].

The Dam1 and Ndc80 complexes are crucial substrates of Aurora B/Ipl1 at kinetochores [30,36,91,92]. Dam1 is primarily phosphorylated at its C-terminus and mutants mimicking constitutive dephosphorylation show defects in bi-orientation. Recent structural studies revealed that the C-terminus of Dam1 protein could affect both oligomerization to form rings and microtubule interaction (Figure 3d) [73] (also refer to a biochemical study [93]). Intriguingly, Dam1 mutants mimicking constitutive phosphorylation reduced the efficiency of ring formation in vitro [73]. On the contrary, phosphorylation of the Ndc80 complex is clustered at the N-terminus of the Ndc80 subunit extending out of the CH domain that is important for microtubule binding (Figure 2a and c). Phosphorylation of this region reduced affinity of the Ndc80 complex for microtubules in vitro [30] and Ndc80 mutants mimicking constitutive dephosphorylation showed defects in bi-orientation in vivo [36]. Thus, the functional consequences of Aurora B phosphorylation on the Dam1 and Ndc80 complexes have been revealed both in vitro and in vivo.

Once bi-orientation occurs and tension is applied on kinetochores, turnover of kinetochore–spindle pole connections must stop [5,94]; otherwise bi-orientation would never be maintained. For this, sensing tension is of central importance, but which component acts as a tension sensor? Bir1 and Sli15 (Survivin and INCENP in metazoans) are binding partners of Ipl1 in yeast and regulate its kinase activity [95]. It was recently revealed that Bir1 and Sli15 form a subcomplex, which forms bridges between a microtubule and a kinetochore [96]. Bir1-Sli15 is therefore positioned ideally to sense tension and may regulate Ipl1 kinase activity accordingly. In metazoans, another good candidate for a tension sensor might be PICH, a Smc2 family member, identified in mammalian cells. PICH shows a unique thread-like localization between bi-oriented sister kinetochores and is necessary for activation of the spindle-assembly checkpoint [97]; considering its localization, PICH may work as a tension sensor to regulate both checkpoint signaling and bi-orientation.

The role of Aurora B/Ipl1 in bi-orientation was initially highlighted in budding yeast [84,85,87] where only one microtubule attaches to each kinetochore [62]. However, in metazoan cells, multiple microtubules form end-coupled attachments to each kinetochore [98]. Thus, Aurora B may have a more complex role in ensuring bi-orientation in this context. Consistent with this idea, inactivation of Aurora B leads to not only syntelic attachment defects but also frequent merotelic attachments [99,100], where a single sister kinetochore attaches to microtubules extending from opposite spindle poles. In addition to its role in promoting turnover of kinetochore–microtubule attachments, Aurora B (together with Polo kinase) facilitates resolution of sister chromatids in metazoan cells [101]; both functions may be important to avoid or correct merotelic attachment.

Mps1 is another evolutionarily conserved protein kinase, required for the spindle assembly checkpoint and, in
some organisms, for duplication of microtubule-organizing centres [102]. Separately from these functions, however, Mps1 has an important role in chromosome segregation [103]. It was recently shown that, in budding yeast, Mps1 has a crucial role in establishing sister kinetochore bi-orientation on the mitotic spindle [104]. Similarly to Ipl1, Mps1 promotes re-orientation of kinetochore–spindle pole connections and eliminates those that do not generate tension between sister kinetochores. Intriguingly, both Ipl1 and Mps1 phosphorylate the Dam1 subunit of the Dam1 complex, but at different sites [91,105]. The role of Mps1 in bi-orientation needs to be investigated further.

Conclusion and perspectives
Over the past couple of years, the kinetochore–microtubule attachment interface has been revealed in increasing detail, in particular, through studies of the Ndc80 and Dam1 complexes. These discoveries have shed new light on the mechanisms underlying kinetochore motion and bi-orientation. Biochemical reconstitutions, structural analysis, genetics and cell biology have all contributed to these discoveries and will continue to advance research in this field.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors quantify the copy number of each kinetochore component at a single centromere in budding yeast. They compared the signal intensity
of GFP-tagged kinetochore components with that of GFP-tagged Cse4, a centromeric histone H3 variant. In budding yeast, a centromere is specified by a single nucleosome [106] containing two copies of Cse4. The authors also perform photobleaching studies that revealed that the Dam1 complex and the Ndc80 complex are stably bound in metaphase.

30. Cheeseman IM, Chappe JS, Wilson-Kubalek EM, Desai A: The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 2006, 127:963-957. The authors biochemically reconstitute KNL-1, the Ndc80 complex and the Mis12 complex and show that they directly associate to form a larger complex, named the KMN network, which possesses two microtubule-binding domains: one in the globular Ndc80/Nuf2 heterodimers and the other in KNL-1. Phosphorylation of the N-terminal extension of the Ndc80 subunit by Aurora B reduced affinity of the Ndc80 complex for microtubules. Reconstitution of the network resulted in a significant enhancement of microtubule-binding affinity relative to the individual microtubule-binding domains.

31. Wei RR, Al-Bassam J, Harrison SC: The Ndc80/HEC1 complex is a contact point for kinetochore–microtubule attachment. Nat Struct Mol Biol 2007, 14:54-59. The authors report the crystal structure of the most conserved region of the Ndc80 globular domain and conclude that it forms a calponin-homology domain. They further show that an Ndc80-Nuf2 heterodimer binds the microtubule lattice in vitro. The N-terminal 80 amino acids of Ndc80, not present in the structure, contribute significantly to the binding affinity.


35. DeLuca JG, Gall WE, Ciferri C, Cimini D, Musacchio A, Salmon ED: Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 2006, 127:969-982. The authors report that microinjection of an antibody against the N-terminus of Ndc80 suppresses the dynamic behaviour of microtubules at kinetochores in Ptk1 cells. They also find that the N-terminus of Ndc80 is phosphorylated by Aurora B kinase in vitro. Expression of non-phosphorylatable Ndc80 causes frequent merotelic attachment and chromosome mis-segregation.


37. Dong Y, Vanden Beldt KJ, Meng X, Khodjakov A, McEwen BF: The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. Nat Cell Biol 2007, 9:516-522. Using electron tomography, the authors visualized the kinetochore-microtubule interface in high resolution, in metaphase of Ptk1 cells. Within the kinetochore outer plate, distinct unit motifs are not found; instead, several fibres either embed the microtubule plus-end in a radial mesh or extend out to bind microtubule walls.


39. Tanaka K, Kitamura E, Kitamura Y, Tanaka TU: Molecular mechanisms of microtubule-dependent kinetochore transport toward spindle poles. J Cell Biol 2007, 178:269-281. The authors reveal two distinct mechanisms, by which kinetochores are transported by microtubules towards spindle poles after they are captured by microtubule lateral surface in prophase of budding yeast. Kar3, a kinesin-14 member, drives kinetochore sliding along the microtubule lateral surface, while the Dam1 complex promotes motion of kinetochores, tethered at microtubule ends (end-coupled pulling). Sliding is often converted to end-coupled pulling but the opposite conversion is rare.


45. Westermann S, Wang HW, Avila-Sakar A, Drubin DG, Nogales E, Barnes G: The Dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends. Nature 2006, 440:565-569. Using in vitro reconstitution, the authors show that Dam1 complexes accumulate at the ends of depolymerising microtubules, processively follow a shrinking microtubule end and can couple movement of a bead to the depolymerizing microtubule. When Dam1 complexes bind along a microtubule, they exhibited one-dimensional diffusion.


49. Franck AD, Powers AF, Gestard DR, Gonen T, Davis TN, Ashby CL: Tension applied through the Dam1 complex promotes microtubule disassembly providing a direct mechanism for length control in mitosis. Nat Cell Biol 2007, 9:832-837. In these two papers, using optical tweezers, the authors measured the amount of tension that can be resisted by a Dam1-complex-coated beads localizing at the microtubule plus ends [49]. Higher tension decreases the frequency of microtubule catastrophe and increases that of microtubule rescue [50], similarly to the effects on kinetochore microtubules during metaphase in vivo.


52. Kitamura E, Tanaka K, Kitamura Y, Tanaka TU: Kinetochore–microtubule interaction during S phase in Saccharomyces cerevisiae. Genes Dev 2007, 21:3319-3330. The authors show that in budding yeast, which shows closed mitosis, DNA replication of centromeres causes their detachment from microtubules, thus displacing them away from a spindle pole for 1–2 min. Soon afterwards kinetochores are reassembled, leading to their recapture by microtubules and subsequent transport towards a spindle pole.


Kinesin-13s are involved in the regulation of mitotic spindle dynamics. In these two papers, the authors show that kinesin-13 proteins are required for the formation of rings and spirals around microtubules. The authors identified the Skl1/2 complex in mammalian cells, which associates with kinetochores following microtubule attachment in prometaphase. Depletion of Skl1/2 shows metaphase arrest due to spindle-checkpoint activation.


The authors identified Bod1 protein through proteomic analysis of chromosome-microtubule attachment and reduces MCAK phosphorylation without an appreciable change in Aurora B activity or localization.


By inhibiting ZW10 that is required for dynein association with kinetochores, the authors studied the roles of dynein at kinetochores in mammalian cells. Dynein at kinetochores is required for rapid poleward chromosome motion in prometaphase and chromosome congression in metaphase. Moreover, ZW10 depletion leads to a reduced velocity of anaphase chromosome motion without affecting microtubule poleward flux.

King JM, Hays TS, Nicklas RB: Dynein is a transient kinetochore component whose binding is regulated by microtubule attachment, not tension. J Cell Biol 2000, 151:739-748.


By using live-cell imaging and correlative electron microscopy, the authors found that mono-oriented chromosomes could glide towards the spindle equator (metaphase plate) along the microtubule microtubule bundles of others, already bi-oriented chromosomes. This gliding is dependent on the kinetochore-associated plus-end-directed motor protein CEP-N.


The authors showed that Kip1/Cin8 (kinetochore-5) and Kip3 (kinetochore-8) are associated with kinetochores during metaphase in budding yeast. Kip3 (kinetochore-14) is at kinetochores in prometaphase [40], but its amount decreases in metaphase. Cin8 and Kip1 regulate kinetochore positions on the metaphase spindle while Kip3 regulates poleward kinetochore motion in anaphase A.


62. Cell structure and dynamics


Using a biochemical reconstitution approach, the authors found that a Bir1-Sli15 complex links budding yeast centromeres to microtubules. This linkage does not require Ipl1 kinase, whose targeting and activation is regulated by Bir1 and Sli15. Elimination of the Bir1-Sli15 linkage, for example, by deletion of the microtubule-binding domain of Sli15 leads to a defect in bi-orientation, similarly to ipi1 mutants.


The authors identify PICH as a Plk1 (Polo-like kinase 1) binding protein and its substrate. Depletion of PICH abrogated the spindle checkpoint. PICH localizes along threads between sister kinetochores during metaphase, which diminish during anaphase. The PICH-positive threads are sensitive to DNase and exacerbated by inhibition of topoisomerase II.


100. Knowlton AL, Lan W, Stukenberg PT: Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. Curr Biol 2006, 16:1705-1710.

These two papers report that inhibition of Aurora B kinase activity leads to frequent occurrence of merotelic kinetochore–microtubule connections in vertebrate cells. In prometaphase, Aurora B inhibition causes suppression of kinetochore–microtubule turnover [99]. Aurora B is enriched at merotelic attachment sites, independently of its kinase activity, and Aurora B enriches MCAK at these sites using its kinase activity [100].


The authors show that Mps1 kinase has a crucial role in establishing sister centromere bi-orientation on the mitotic spindle in budding yeast. This role of Mps1 is separate from its function in the spindle-assembly checkpoint and spindle pole body duplication. Similarly to Ipl1, Mps1 facilitates bi-orientation in a tension-dependent mechanism by eliminating wrong kinetochore–spindle pole connections.

