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Kinetochore-Microtubule Interactions: The Means to the End

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Summary

Kinetochores are proteinaceous complexes 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.

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 [1–3]). For high-fidelity chromosome segregation, kinetochores must capture spindle microtubules and connect the sister chromatids of all chromosomes to opposite spindle poles prior to anaphase onset. Proper kinetochore capture of spindle microtubules is achieved in a stepwise manner (reviewed in [4,5]; Fig 1). Kinetochores initially attach to the surface of a single microtubule that extends from either spindle pole [6–8] (Fig 1, step 1). Once bound, kinetochores are transported poleward along microtubules (Fig 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 (Fig 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 [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 [11]). Then each sister kinetochore, attached to the plus end of microtubules, is pulled towards the opposite spindle poles during anaphase (Fig 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 [12, 13]).

In this article, we review recent papers (over the last 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 [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?

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 [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 (Fig 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 ~55 nm long rod structure with globular domains at both ends. The Spc24/25 globular domain is oriented towards the inner kinetochore whereas the Ndc80/Nuf2 globular domain projects outwards and is positioned to interact with spindle microtubules. In metazoans, the Ndc80 complex associates with kinetochores prior to nuclear envelope breakdown [26,27]. Photobleaching studies indicate that this association is very stable [28,29]. In budding yeast, direct measurements have shown that 5–8 Ndc80 complexes are present per bound single kinetochore microtubule [29]; these observations suggest that multivalent interactions between kinetochore-associated Ndc80 complexes and spindle microtubules is critical for chromosome segregation.

Most significantly, biochemical analysis and electron microscopy of the reconstituted Ndc80 complex has shown that the Ndc80-Nuf2 subunits (in particular, their globular domains)

directly interact with the microtubule lattice, albeit with a low affinity [30,31] (Fig 2B). The crystal structure of the globular domain of the Ndc80 subunit has revealed that it is folded into a calponin-homology (CH) domain (Fig 2A, bottom) [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 critical 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 appear 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 critical 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 interacting protein set termed the KMN network from the names of its constituent parts (reviewed in [3]; Fig 2C). The Mis12 complex does not bind directly to microtubules *in vitro* whereas KNL1 does, albeit weakly and with a high degree of cooperativity [30]; KNL1 family proteins may also bind to microtubules in vivo, as suggested by analysis in fission yeast [37]. Reconstituting the interaction of these three pieces by mixing purified complexes increases the net microtubule-binding affinity, either via generation of a locally high density of microtubule-binding sites or via allosteric regulation [30]. Thus, in vivo, the Ndc80 complex is acting in the context of this protein set, which not only directs its localization but may also influence its microtubule-binding activity. Based on these findings, it was proposed that microtubule ends are bound by a network composed of the KMN protein group [30]. Consistent with this proposal, electron tomography has recently shown that the microtubule ends are embedded in a fibrous network within the kinetochore outer plate [38].

Dam1 Complex Rings: Force Transducers for Pulling on Kinetochores

During end-coupled attachments, a special device is necessary to maintain kinetochore association with dynamic microtubule ends. Recent results suggest that the Dam1 complex has the properties to construct such a device. The Dam1 complex, also called DASH or DDD, is composed of at least 10 proteins, and has been identified in yeast (reviewed in [15]). This

complex is not at kinetochores during their initial association with the microtubule lateral surface; however it subsequently is present at end-coupled attachments [39,40], possibly because of a direct Dam1 complex—Ndc80 complex interaction [41–43]. The Dam1 complex plays a crucial role in tethering kinetochores at the ends of depolymerizing microtubules in budding yeast [40]. Biochemical reconstitution has revealed that several (~16) Dam1 complexes associate together on the microtubule surface to form a ring that encircles the polymer [44,45] (Fig 3A). If this view is correct, Dam1 complex rings formed along a microtubule should accumulate at the microtubule plus end during the outward curling of protofilaments that accompanies microtubule depolymerization (Fig 3B); this was indeed the case not only in an *in vitro* reconstituted system [46], but also in *in vivo* observations [40].

I*n 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 [40,51–53] (end-coupled pulling; Fig 1, step 2, right; Fig 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 kinetcohore [12,13,54] (Fig 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: first, 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 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], 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 (orthologs 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 [64–69], similar to Dam1 (see below),

and can form rings/spirals encircling a microtubule at least *in vitro* [70–72]. 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 [74], 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 [75], 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 [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; Fig 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; Fig 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 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) [10]. It has been thought that congression occurs after, and depends on, sister kinetochore bi-orientation. However, in metazoan cells prior to bi-orientation, kinetochore-bound CENP-E, a kinesin-7 family plus end-directed motor, facilitates this process by transporting mono-oriented kinetochores away from a spindle pole along microtubules that are attached to other already congressed and bi-oriented kinetochores [82]. Perhaps analogously, in budding yeast, Cin8, a kinesin-5 family member, localizes at bi-oriented kinetochores and regulates kinetochore position on the metaphase spindle [83]

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 prior to 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 (Fig 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 other hand, 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 (Fig. 2A, 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 metazoa) are binding partners of Ipl1 in yeast and regulate its kinase activity [95]. It was recently revealed that Bir1 and Sli15 form a sub-complex, 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 Snf2 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 last 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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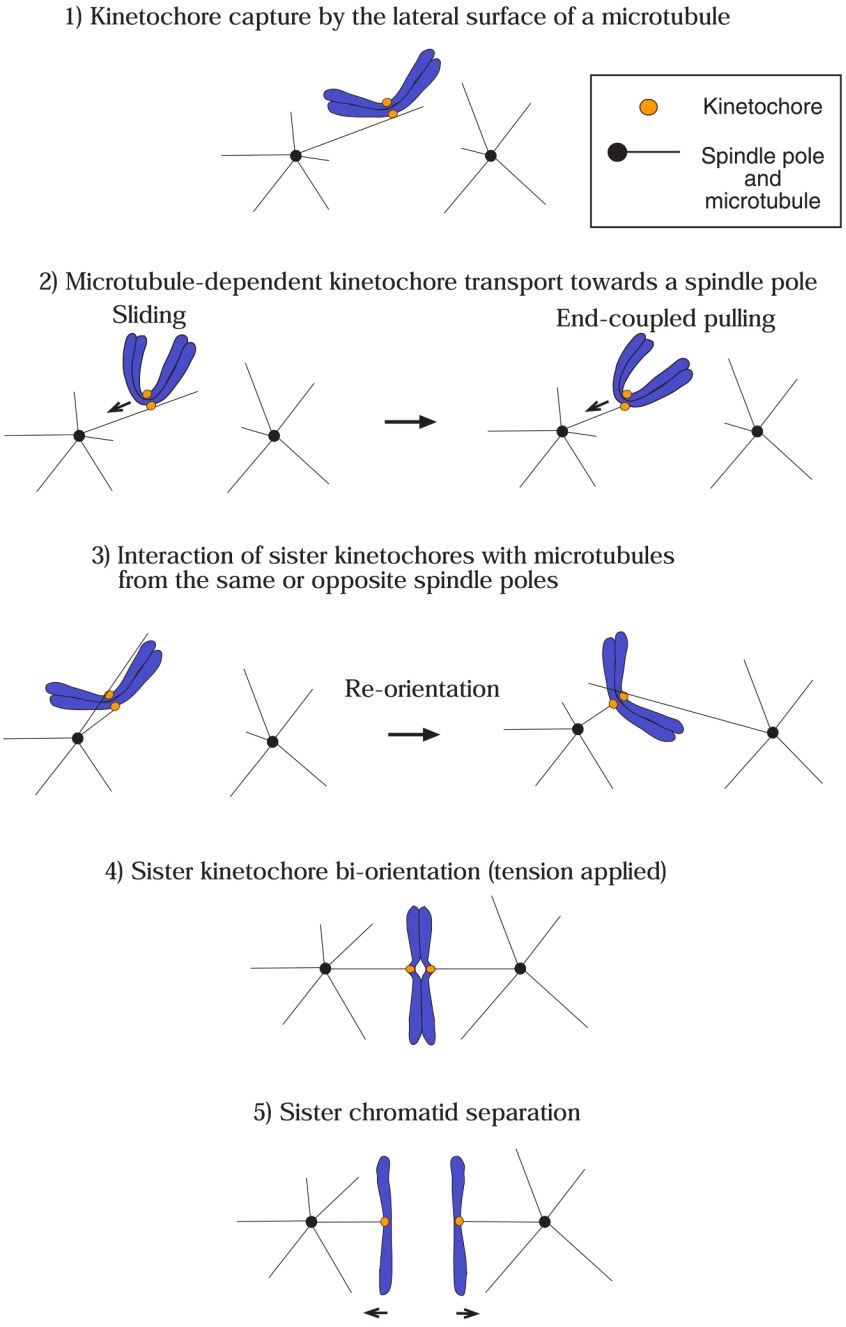

Figure 1. Overview of kinetochore-microtubule interactions

The figure depicts kinetochore-microtubule interactions during prometaphase (steps 1–3), metaphase (step 4) and anaphase A (step 5).

1) 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 step 1–2) [53].

2) Once captured, kinetochores are transported along the lateral surface of single microtubules toward 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) [40,53].

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].

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].

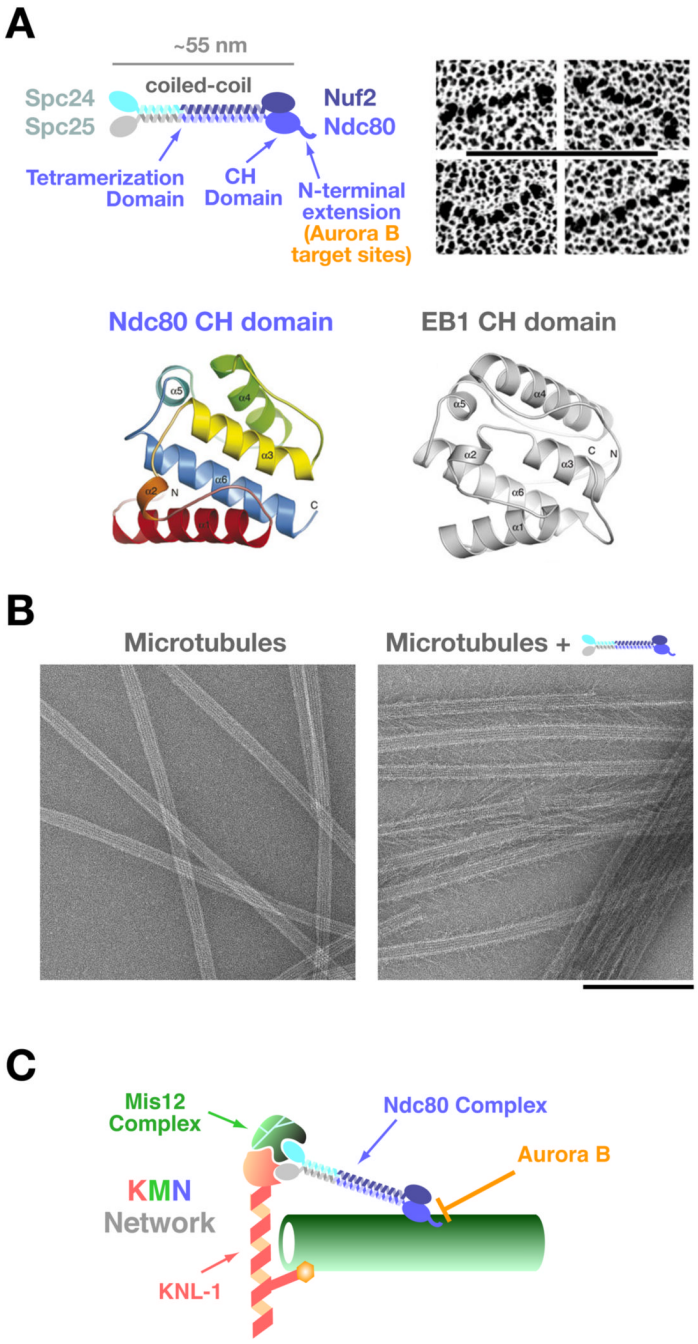

Figure 2. The Ndc80 csomplex and the KMN network

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 ref. [23]). 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 [31]).

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 [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].

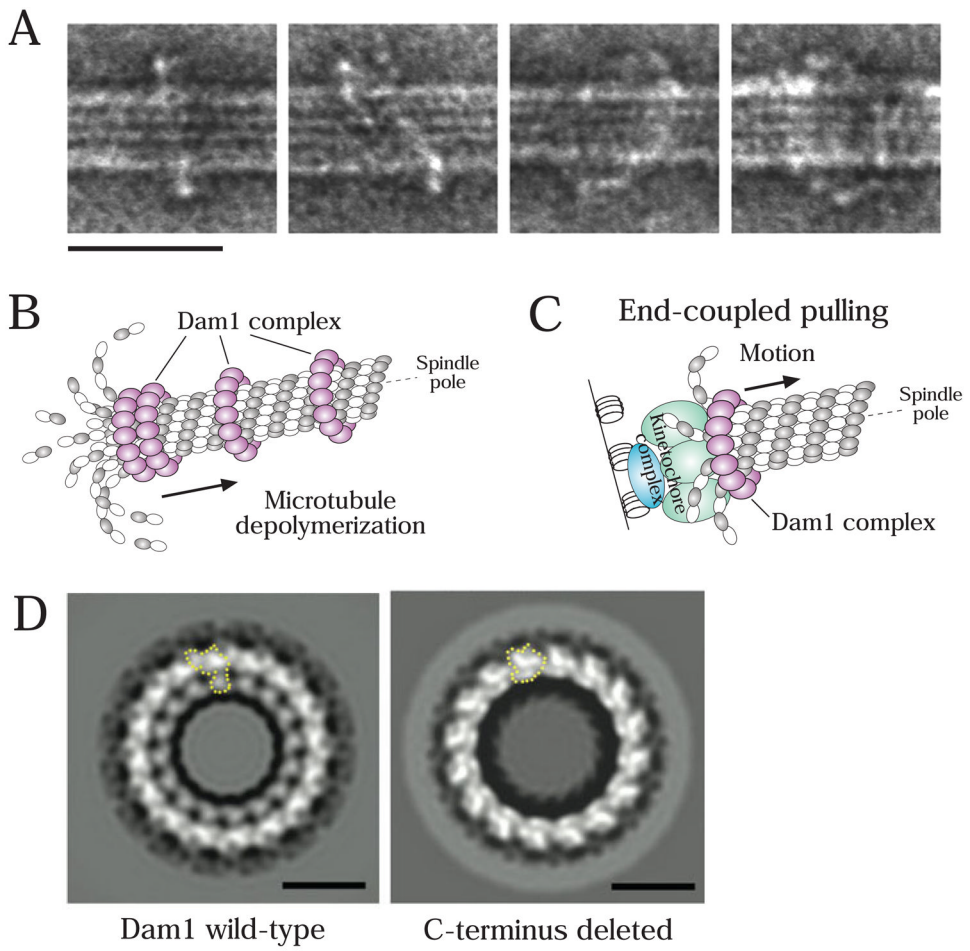

Figure 3. Dam1 complexes oligomerize to form a ring encircling a microtubule

A) Electron micrographs of negatively stained microtubules in the presence of the Dam1 complexes. Scale bar, 50 nm. Reprinted from [44].

B) If the Dam1 complexes formed rings around microtubules, they would be accumulated at the microtubule plus end during the outward curling of protofilaments that accompanies depolymerization; this accumulation was observed *in vitro* [46] and *in vivo* [40].

C) The Dam1 complex has a crucial role in tethering kinetochores at microtubule ends and in converting microtubule depolymerization into kinetochore pulling force [40,46,49].

D) Electron micrographs of rings (negative stain and 16-fold rotational average), formed by the Dam1 complexes containing either a wild-type Dam1 protein (left) or a C-terminus (140 residues)-deleted Dam1 protein. A single Dam1 complex is shown by the dotted line. Scale bars, 25 nm. Reprinted from [73].