A blueprint for kinetochores — new insights into the molecular mechanics of cell division

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Abstract | Kinetochores are large proteinaceous complexes that physically link centromeric DNA to the plus ends of spindle microtubules. Stable kinetochore–microtubule attachments are a prerequisite for the accurate and efficient distribution of genetic material over multiple generations. In the past decade, concerted research has resulted in the identification of the individual kinetochore building blocks, the characterization of critical microtubule-interacting components, such as the NDC80 complex, and the development of an approximate model of the architecture of this sophisticated biological machine.

The constitutive structural and regulatory elements of the eukaryotic kinetochore are conserved throughout evolution, suggesting that there is a canonical protein framework that differs only in the level of complexity between humans and yeast[1,2] (FIG. 1a; TABLE 1). Kinetochore assembly is restricted to centromeres, which are chromosomal domains that are specified by the presence of nucleosomes that contain the histone H3 variant centromeric protein A (CENPA; known as chromosome segregation 4 (Cse4) in *Saccharomyces cerevisiae*). Budding yeast has point centromeres of defined length (~125 bp) and sequence, on which a single Cse4 nucleosome is positioned[3]. By contrast, centromeres of human chromosomes span megabases and contain arrays of tandemly repeated α-satellite DNA sequences, CENPB boxes that are bound by the inner centromere protein CENPB, and multiple copies of CENPA. Notably, each kinetochore in budding yeast is connected to only a single spindle microtubule, whereas the human kinetochore provides a platform for the attachment of a fibre consisting of multiple microtubules.

CENPA nucleosomes associate with the conserved DNA-binding protein CENPC (known as Mif2 in budding yeast), which is part of the constitutive centromere-associated network of proteins (CCAN). The Ctf19 (chromosome transmission fidelity 19) complex in budding yeast is the functional equivalent of the human CCAN. CCAN components are required for the recruitment of the conserved linker complexes of the KMN network: the KNL1 complex (known as the Spc105 (spindle pole body component 105) complex in yeast), the MIS12 complex (known as the Mtw1 complex in yeast) and the NDC80 complex.

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The KMN network forms a bridge between the centromere-associated proteins and the plus ends of spindle microtubules[4]. Within the KMN network, the NDC80 complex and the KNL1 complex build a composite microtubule-binding site and, *in vitro*, the NDC80 complex has been shown to support load-bearing microtubule attachments[5,6]. In all eukaryotes, depletion of any KMN component leads to an aberrant kinetochore structure and, in the worst case, to a complete lack of kinetochore–microtubule attachments[7–9].

Budding yeast kinetochores differ from this general organization principle in two elements (FIG. 1b). First, the sequence-specific DNA-binding complex Cbf3 is required for Cse4 deposition, and thus the initiation of kinetochore assembly. Second, efficient chromosome segregation in budding yeast requires the ten-protein complex Dam1 (Duo1- and Mps1-interacting 1), which is loaded onto kinetochores in an Ndc80- and microtubule-dependent manner[10–12]. An attractive explanation for the specific requirement for the Cbf3 and Dam1 complexes in budding yeast is that they represent adaptations of the kinetochore structure to the budding yeast-specific point centromere and the single microtubule attachment site, respectively. However, there is probably a requirement in all other eukaryotes for additional proteins to aid the formation of a functional kinetochore–microtubule interface. In humans, the recently discovered spindle and kinetochore-associated (SKA) complex, which was shown to be crucial for robust spindle microtubule attachments, may carry out this function[13,14]. However, it is not yet clear how the SKA complex topologically and functionally integrates into the kinetochore, and it is therefore not discussed further in this Progress article.

In both human and budding yeast cells, kinetochore–microtubule attachments are regulated by the conserved Aurora B kinase (known as Ipl1 in budding yeast). Aurora B activity towards key downstream targets, such as NDC80 and Dam1, prevents the premature stabilization of attachments that fail to produce tension across sister chromatids[15]. It is thought that Aurora B, as part of the chromosome-passenger complex (CPC), is enriched at the inner centromere and that upon sister chromatid biorientation (that is, the attachment of sister chromatids to opposite spindle poles), key substrates are spatially separated from the active kinase through tension[16].

In this Progress article, we highlight recent work providing molecular insights into the budding yeast and human kinetochore machinery, with particular emphasis on the NDC80 complex, which has emerged as the essential kinetochore component for

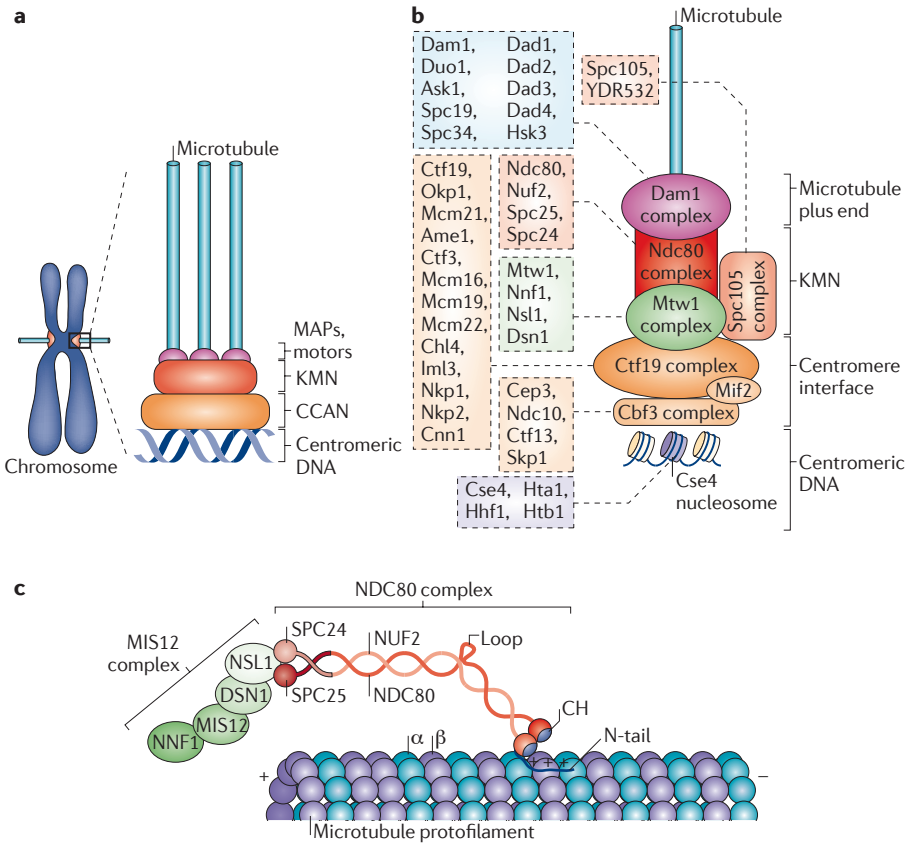

Figure 1 | **General and budding yeast kinetochore organization. a** | Schematic of the general organization of the kinetochore. The main components are the two multi-complex structures, the KMN network (which comprises the KNL1 (known as Spc105 (spindle pole body component 105) in yeast), MIS12 (known as Mtw1 in yeast) and NDC80 complexes) and the constitutive centromere-associated network (CCAN). Only the structural components of the kinetochore are depicted, and this basic structure interacts with regulatory components, such as Aurora B kinase, microtubule-associated proteins (MAPs), motors and components of the mitotic checkpoint machinery (not shown). **b** | The organization of the budding yeast kinetochore and the subunits that make up each complex. The KMN network is at the core of the structure. Towards the microtubule plus end, it cooperates with the ten-protein Dam1 (Duo1- and Mps1-interacting 1) complex to form an attachment site for dynamic microtubules. It is anchored to the centromere through interactions with the Ctf19 (chromosome transmission fidelity 19) complex (which is equivalent to the human CCAN network) and the centromeric protein C (CENPC) homologue Mif2. The budding yeast-specific Cbf3 complex binds centromeric DNA in a sequence-specific manner and may be required to load the single chromosome segregation 4 (Cse4) nucleosome onto the centromere. **c** | Detailed view of the human NDC80 complex. The NDC80 and NUF2 subunits contain calponin homology (CH) domains, which are part of the microtubule-binding interface. In addition, the amino terminus of NDC80 (the N-terminal tail (N-tail)) is required for microtubule binding and kinetochore function. The coiled-coil shaft of the molecule is interrupted by an evolutionarily conserved loop. The SPC24 and SPC25 end of the complex projects towards the inner centromere and physically interacts with the MIS12 complex through the DSN1–NSL1 heterodimer. α, α-tubulin; β, β-tubulin; Ask1, associated with spindles and kinetochores 1; Chl4, chromosome loss 4; Cnn1, co-purified with NNF1 1; Dad, Duo1- and Dam1-interacting; Duo1, death upon overproduction 1; Hhf1, histone H4 1; Hsk3, helper of ASK1 3; Hta1, histone H2A.1; Htb1, histone H2B.1; Iml3, increased minichromosome loss 3.

effective spindle microtubule attachments in all eukaryotes. We first explain how the NDC80 complex, as a central component of the KMN network, is connected to the inner centromere, and then discuss how it interacts with microtubules. Finally, we discuss how a novel strategy to isolate functional kinetochores from yeast extracts can provide a new tool to understand the regulatory and biophysical mechanisms of microtubule attachment and force generation.

The centromere–kinetochore link

Structural and biochemical studies have revealed the overall topology of the kinetochore and have shown that the NDC80 complex is linked to the centromere through interactions with the MIS12 complex and CCAN components.

Clues from the structure of the NDC80 complex. Early structural work on the NDC80 complex laid the groundwork for recent biochemical multicomplex reconstitution experiments that revealed the overall organization of the core kinetochore. The four-protein NDC80 complex is a 57 nm elongated molecule with two globular domains that are separated by an α-helical coiled-coil shaft[17–19] (FIG. 1c). The complex essentially operates as a bifunctional linker: the SPC24–SPC25 globular domain is anchored to the NSL1 subunit of the MIS12 complex within the inner kinetochore, and the NDC80–NUF2 globular domain physically contacts the plus ends of spindle microtubules. Extending this model, high-resolution *in vivo* imaging has shown that the carboxyl terminus of SPC24 is within 45±6 nm of the amino-terminal end of NDC80, which is crucial for microtubule binding[20]. This observation suggests either that the NDC80 complex is not precisely oriented perpendicularly along the microtubule axis or, alternatively, that the NDC80 complex is kinked *in vivo*, reflecting some degree of intramolecular flexibility. Indeed, the coiled-coil shaft is interrupted by an evolutionarily conserved loop insertion that can operate as a flexible hinge and allows the NDC80–NUF2 head to bend relative to the coiled-coil[21]. The hinge is essential for the function of the complex and it may act as a binding site for microtubule-associated proteins[22,23]. A detailed molecular understanding of how the two functional ends of the NDC80 complex interact with their binding partners is crucial for an understanding of kinetochore architecture.

Structural properties of the Mtw1 complex. An important step towards understanding kinetochore architecture was the successful *in vitro* reconstitution of the budding yeast Mtw1 complex and the human MIS12 complex[24–26]. Despite relatively low sequence conservation from yeast to humans, the complex displays conserved structural properties. Its four subunits associate with a 1/1/1/1 stoichiometry and can be assembled from two stable heterodimers: Mtw1–Nnf1 and Dsn1–Nsl1. Electron microscopy (EM) analysis in yeast shows that the complex adopts an elongated dumbbell shape with a length of 22–25 nm and a diameter of 8 nm[24,25]. Immuno-EM localization analysis of N-terminal and C-terminal fusion

Table 1 | **Human and yeast kinetochore components**

Kinetochore complex or associated module*	Human proteins‡	Budding yeast proteins	Comments
NDC80	NDC80 (HEC1), NUF2, SPC24, SPC25	Ncd80, Nuf2, Spc24, Spc25	Part of the KMN network
MIS12 (Mtw1)	MIS12, NNF1, NSL1, DSN1	Mtw1, Nnf1, Nsl1, Dsn1	Part of the KMN network; also referred to as the MIND complex
KNL1 (Spc105)	KNL1 (Blinkin), ZWINT1	Spc105, YDR532	Part of the KMN network
CENPA	CENPA	Cse4	Replaces histone H3 in centromeric nucleosomes
CENPC	CENPC	Mif2	Closely associates with CENPA; can be regarded as a CCAN component
CCAN (Ctf19)	CENPH, CENPI, CENPK, CENPL, CENPM, CENPN, CENPO, CENPP, CENPQ, CENPR, CENPS, CENPT, CENPU, CENPW, CENPX	Ctf19, Okp1, Mcm21, Ame1, Ctf3, Mcm16, Mcm19, Mcm22, Chl4, Iml3, Nkp1, Nkp2, Cnn1	Evolutionary conservation of individual CCAN subunits between human and yeast is not firmly established; Ctf19, Okp1, Mcm21 and Ame1 form a subcomplex known as the COMA complex
Dam1 complex	NA	Dam1, Duo1, Ask1, Spc19, Spc34, Dad1, Dad2, Dad3, Dad4, Hsk3	Seems to be fungus-specific but is not essential in *Schizosaccharomyce pombe*; also referred to as the DASH or DDD complex
SKA complex	SKA1–SKA3	NA	Suggested to be a functional Dam1 complex homologue[14]
Cbf3	NA	Cep3, Ndc10, Ctf13, Skp1	Sequence-specific DNA-binding complex required for Cse4 deposition in budding yeast.

Restricted to components that are discussed in the main text. Ask1, associated with spindles and kinetochores 1; CCAN, constitutive centromere-associated network; CENP, centromeric protein; Chl4, chromosome loss 4; Cnn1, co-purified with NNF1 1; Cse4, chromosome segregation 4; Ctf, chromosome transmission fidelity; Dad, Duo1- and Dam1-interacting; Dam1, Duo1 and Mps1-interacting 1; DASH, Dam1–Duo1–Ask1–Spc34–Spc19–Hsk1; DDD, Duo1–Dam1–Dad1; Duo1, death upon overproduction 1; HEC1, highly expressed in cancer 1; KMN, KNL1–MIS12–NDC80; MIND, Mtw1 including Nnf1–Nsl1–Dsn1; NA, not applicable; Nkp, non-essential kinetochore protein; SKA, spindle and kinetochore-associated; SPC, spindle pole body component; ZWINT1, ZW10-interacting 1. *When different, the yeast complex names are provided in brackets. ‡Alternative names for proteins are provided in brackets.

proteins showed that all four yeast subunits contribute to the organization of the globular domain, whereas the rod may be primarily formed by the predicted coiled-coil domains of Nnf1 and Mtw1 (REF. 25). Similarly to its yeast counterpart, the human MIS12 complex is elongated with a 22-nm-long axis, but has more of a 'beads on a string' appearance[26]. Cross-linking experiments indicate that the DSN1–NSL1 heterodimer of the MIS12 complex is proximal to the SPC24–SPC25 head of the NDC80 complex, whereas MIS12–NNF1 may be distal and provide contacts with the inner centromere. The elongated shape of the MIS12 complex and the location of its binding site at one end of the NDC80 complex heterotetramer demonstrate that the combination of these two complexes can bridge the distance between the microtubule plus end and the inner kinetochore.

The KMN network forms the kinetochore core. Biochemical experiments using recombinant *Caenorhabditis elegans* proteins have demonstrated that the NDC-80 complex and the MIS-12 complex, together with KNL-1, form the central KMN network, which constitutes the inner architectural core of all kinetochores[6]. Extending this work, the budding yeast Ndc80 and Mtw1 complexes have been reconstituted into a higher-order assembly with a 1/1 stoichiometry. Full reconstitution of the budding yeast KMN network is still awaited, as full-length recombinant Spc105 has proven difficult to express. Peptide interaction studies, however, indicate that the C-terminal part of Spc105 is likely to bind the Mtw1 complex[25] in a manner that is non-competitive with the Ndc80 complex. In *C. elegans* the interaction of the NDC-80 and MIS-12 complexes requires KNL-1, whereas in yeast and in humans this association forms autonomously[6,24–26].

A parallel study on the human KMN network reveals an overall conserved building plan, with deviations in specific interaction surfaces: the MIS12 and NDC80 complexes strongly interact through the SPC24–SPC25 head of the NDC80 complex and the DSN1–NSL1 heterodimer of the MIS12 complex. The C terminus of NSL1 is crucial for binding both SPC24–SPC25 and the C terminus of KNL1. Together, the MIS12 complex tied to the C terminus of KNL1 and the NDC80 complex form a tight supercomplex (with an apparent dissociation constant of 4 nM) that comprises all members of the human KMN.

In light of modular kinetochore assembly, the next important question is how the KMN network is tethered to centromeres. In yeast there is evidence for a physical interaction between the Mtw1 complex and the four-protein complex COMA (Ctf19–Okp1–Mcm21–Ame1), which is a biochemically stable subcomplex of the Ctf19 complex[24]. This finding may be of general significance, as localization dependency experiments in human cells implicate members of the CCAN in the recruitment of the MIS12 and KNL1 complexes to the inner centromere[27,28]. In addition, there is evidence for direct binding of CENPC to the MIS12 complex in humans and *Drosophila melanogaster*[29,30]. Furthermore, *in vitro* experiments suggest that during interphase the NSL1 subunit of the human MIS12 complex binds to HP1α (also known as CBX5), which is a component of centromeric heterochromatin[31]. Therefore, it is possible that HP1α contributes to the loading of the MIS12 complex onto kinetochores; however, it is also known that the MIS12 complex switches its binding partner during mitosis, as SPC24–SPC25 binds the same site on NSL1 with higher affinity than HP1α[26]. The question of how the KMN network is connected to DNA will be a major line of future research. It is unlikely that the interaction dependencies are strictly linear and, indeed, the KMN network might be anchored to the inner centromere by multiple means.

The NDC80–microtubule interface

When the centromere-binding proteins are recruited, outer kinetochore proteins create a binding site to host dynamic microtubule plus ends. The requirement to withstand forces able to move chromosomes and, at the same time, be flexible enough to incorporate dynamic microtubule instability without losing attachments is a unique property of the physical engagement of the kinetochore with spindle microtubules.

Binding of the NDC80 complex to microtubules. Work from the Harrison and Musacchio[17,18] laboratories first described structural elements in the NDC80–NUF2 head of the NDC80 complex that directly contribute to the intrinsic microtubule-binding activity of the complex. The N-terminal regions of NDC80 and NUF2 fold into a globular head harbouring two interacting calponin homology (CH) domains (FIG. 1c). This was a functionally important revelation, as CH domains are also present in the prototypical human microtubule plus end-binding protein EB1 (also known as MAPRE1)[32], and indicates that the N-terminal regions of NDC80 and NUF2 form a composite microtubule-binding interface. Mutations in both the NDC80 and NUF2 CH domains significantly lower the affinity of the NDC80 complex for microtubules *in vitro*[17], and a functional CH domain is essential for chromosome segregation in human cells[33,34] and in budding yeast (F.L. and S.W., unpublished observations). The CH domains, however, are only one half of NDC80's bipartite microtubule-interaction domain, as they are combined with an intrinsically unstructured, highly positively charged region located at the N terminus of NDC80 (the N-terminal tail (N-tail)). Deletion of the N-tail of NDC80 reduces its affinity for microtubules *in vitro* and leads to a complete loss of kinetochore–microtubule attachments in human cells[35,36]. The N-tail is also an important regulatory element of the complex, as it acts as a target for multisite phosphorylation by Aurora B, which prevents erroneous kinetochore–microtubule attachments by changing the electrostatic properties of this positively charged region[36].

Cryo-EM reconstructions of microtubules decorated with *C. elegans* NDC-80–NUF-2 (REF. 37), or with an artificially shortened version of the human NDC80 complex[38], showed that the NDC80 complex binds both α-tubulin and β-tubulin with a periodicity of 4 nm. NDC80 complexes bind the lateral surface of the microtubule at a defined angle and leave the plus end accessible for addition or removal of tubulin dimers. The subnanometer resolution of this reconstruction allowed docking of the known crystal structure of the NDC80–NUF2 dimer, revealing that the contact area between NDC80 and microtubules is formed by a limited region within the NDC80 CH domain — the 'toe' — and a highly conserved binding site present at intra-dimer and inter-dimer microtubule interfaces — the 'toeprint' (REF. 38). Thus, the NDC80 complex uses a minimal surface area to contact tubulin monomer repeats. The CH domain of NUF2 does not directly contact the microtubule surface but is positioned to electrostatically interact with the negatively charged, flexible C terminus of tubulin (known as the E-hook domain). Interestingly, under non-saturating conditions, individual NDC80 complexes tend to form clusters on the microtubule lattice, a behaviour that is at least in part mediated by the N-tail of NDC80. Thus, the N-tail of NDC80 may constitute an oligomerization factor, an activity that is suppressed by Aurora B phosphorylation.

> "The N-tail of NDC80 is essential for ... attachments in HeLa cells, but the corresponding budding yeast mutant is not compromised for growth"

NDC80 complex-mediated chromosome movement. The structural analyses of NDC80 complex–microtubule interactions fit well with the diffusion-based hypothesis for chromosome movement that was originally postulated by Hill[39]. This theory states that chromosome movement is generated by a microtubule depolymerization-dependent force that is transduced by multiple weak interactions of kinetochore molecules with the plus end of microtubules. An array of NDC80 complexes could form the 'Hill Sleeve' around the microtubule that is proposed to couple the kinetochore to the microtubules[39]. Microtubule lattice-bound NDC80 complexes diffuse rapidly and bidirectionally, but depolymerization of the plus end of the microtubules biases the diffusion of the complexes towards the minus end, thereby dragging the chromosome to the pole. According to the model predicted from the structure of the NDC80 complex described above, an NDC80 complex with a phosphorylated N-tail may display weakened binding to the microtubule, in a manner that allows the correction of attachment mistakes. On successful kinetochore biorientation, dephosphorylation of the NDC80 N-tail by phosphatases opposing Aurora B could then create high-affinity binding by enhancing the affinity of individual NDC80 complexes for microtubules and also allowing oligomerization. The elucidation of higher-resolution structures of the NDC80 complex decorating microtubules will allow experimental dissection of the proposed model in great detail.

Interaction of the Ndc80 complex with Dam1 in yeast. NDC80 is clearly an essential part of kinetochore–microtubule attachments in all eukaryotes, but there seem to be different functional requirements in human cells compared with budding yeast. The N-tail of NDC80 is essential for chromosome–microtubule attachments in HeLa cells, but the corresponding budding yeast mutant is not compromised for growth[40,41]. Furthermore, when compared directly, the human NDC80 complex outperforms the yeast complex in microtubule-binding activity, which suggests that at least one additional component is required in yeast (F.L. and S.W., unpublished observations).

The prime candidate for an additional critical component of the kinetochore–microtubule interface in budding yeast is the Dam1 complex. Artificial recruitment of the complex to DNA is sufficient to mediate DNA segregation *in vivo*[42,43]. *In vitro*, 16 Dam1 heterodecamers oligomerize into a ring encircling microtubules, a property that highlighted the potential role of the complex as an ideal microtubule-coupling device[44,45]. The complex tracks microtubule tips and can translate the force that is generated by microtubule depolymerization into movement of cargo[46,47]. Interestingly, significantly more Ndc80 complexes are recruited to microtubules *in vitro* in the presence of the Dam1 complex[41]. The Dam1 complex autonomously tracks growing microtubule plus ends, and the Dam1 and Ndc80 complexes physically engage at the microtubule plus end and move with dynamic microtubule tips *in vitro*. Furthermore, this microtubule-specific interaction is disrupted by phosphorylation of the Dam1 complex by Aurora B[41,48].

The ability of Dam1 to engage with the Ndc80 complex *in vitro* does not strictly depend on Dam1 ring formation, leaving open the question of whether this is the physiologically relevant form of the complex. Furthermore, the geometry and precise structure of the Ndc80–Dam1–microtubule arrangement also still unclear

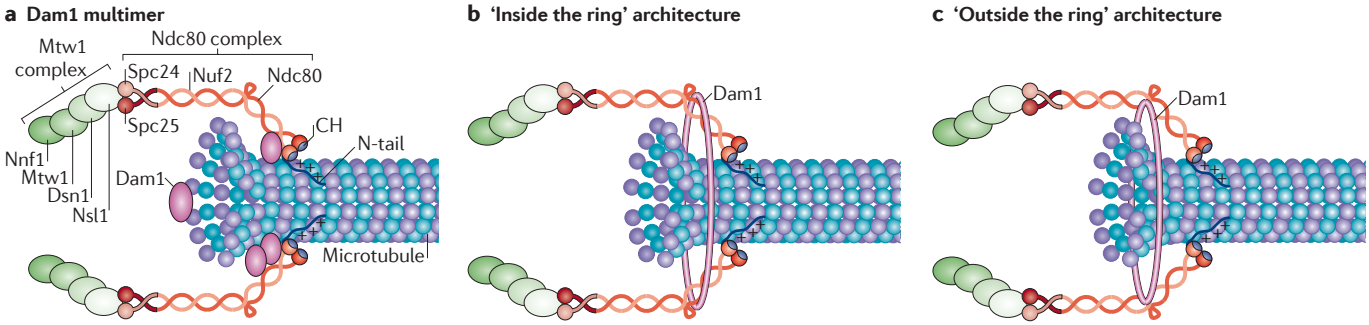

Figure 2 | **Possible configurations of Ndc80 and Dam1 complexes at the yeast kinetochore.** In budding yeast, in which only a single microtubule attaches to each kinetochore, the Dam1 (Duo1- and Mps1-interacting 1) complex has a key role in supporting the function of the Ndc80 complex. Images from high-resolution fluorescence microscopy indicate that Dam1 is closer to the plus end of the microtubule than the Ndc80–Nuf2 head[51]. Different geometric configurations of the Dam1 and Ndc80 complexes are conceivable. **a** | Individual Dam1 complexes or small oligomers may simultaneously bind the microtubule lattice and the Ndc80 complex, thus helping to link Ndc80 to the dynamic microtubule. **b** | Dam1 may oligomerize into a ring that acts as a 'sliding clamp' to enhance the attachment and processivity of the Ndc80 complex. **c** | Ndc80 complexes may interact with the outer surface of a Dam1 ring that is positioned close to the plus end of the microtubule. CH, calponin homology; N-tail, N-terminal tail; Spc, spindle pole body component.

(FIG. 2). A Dam1 ring could act as a 'sliding clamp', forcing the persistent attachment of Ndc80 complexes on a single microtubule. Alternatively, individual Dam1 molecules or small oligomers may simultaneously interact with microtubules and with Ndc80. Visualizing the actual configuration by EM will be necessary to understand the mechanism of microtubule-coupled force generation by the yeast kinetochore. In addition, mutant analysis should help to determine structural elements in both complexes that contribute to the interaction. Why is the Dam1 complex crucial for chromosome segregation in budding yeast but not essential in other yeasts such as *Schizosaccharomyces pombe* and seemingly not conserved outside fungi? A possible explanation is that the single-microtubule configuration makes Dam1 indispensable. One can imagine that, in a kinetochore fibre that consists of multiple microtubules, individual transient detachment events are tolerated as long as a sufficient number of microtubules can provide the attachment to the chromosome. By contrast, when correct biorientation has been achieved in budding yeast, the cell cannot allow detachment of the single microtubule that is attached to each chromosome.

Ensemble properties

Although the detailed work on the NDC80 complex and its interactions has provided important insights into the operation of a key component, a fully assembled kinetochore is likely to have properties that are more than just the sum of its parts. A recent study has reported the successful purification of budding yeast kinetochore particles that contain all major DNA- and microtubule-binding kinetochore proteins in low but detectable amounts[49]. The isolation strategy is based on the affinity-purification of FLAG-tagged Dsn1, which co-purifies with most kinetochore proteins. Interestingly, these particles mimic key aspects of native kinetochores. First, they bind stably to the lateral surface of microtubules, an activity that depends on the Ndc80 and Spc105 complexes but not on the Dam1 complex. Consistent with this, *in vivo*, the Dam1 complex is not required for lateral attachments of kinetochores to microtubules[50]. Second, these particles track microtubule plus ends in a processive manner, persisting at the tip during multiple rounds of tubulin polymerization and depolymerization and withstanding loads in the physiological range of forces operating at kinetochores. Third, an increase in tension prolongs the average lifetime of particle attachments, a property that may reflect the mechanical stabilization of kinetochore–microtubule attachments through bipolar spindle attachments that has been observed *in vivo*.

However, many questions remain: what is the precise molecular composition of the purified kinetochore particles? Can they indeed be regarded as native kinetochores, or are they instead subassemblies that form stably in yeast extracts? It remains possible that centromeric chromatin is strictly required to form kinetochores with the correct combination of DNA-binding and KMN components. What are the stoichiometries of individual complexes and proteins? What glues the complexes together (possibly even in the absence of DNA)? How do post-translational modifications influence the assembly, composition, microtubule binding and biophysical parameters of kinetochores? Regardless of the exact biochemical nature of the FLAG-tagged Dsn1 purified kinetochores, the ability to isolate kinetochore assemblies from yeast extracts, for functional and structural studies at different cell cycle stages, under different checkpoint conditions or in various mutants, will probably yield important new insights in the future.

Conclusion and perspectives

There has been tremendous progress in the understanding of the architectural and biophysical parameters of kinetochore proteins and multicomplex assemblies. Reconstitution experiments have defined the interaction surfaces between individual components, but it remains unclear how assembly into a mature kinetochore, which contains multiple copies of each complex, is achieved. Are the multimerization properties intrinsic to certain kinetochore complexes, or are they dictated by the centromere interface that contains a defined number of KMN receptors? It is unknown how the assembly pathway is regulated during cell cycle progression and what happens to kinetochores on replication of centromeric DNA. Answering these questions is integral when it comes to constructing a functional kinetochore *in vitro*. In the future, the combination of biochemical reconstitutions and structural work promises to create an even more detailed view of the fascinating chromosome segregation machinery.

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