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How kinetochore architecture shapes the mechanisms of its function

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Abstract

The eukaryotic kinetochore is a sophisticated multi-protein machine that segregates chromosomes during cell division. To ensure accurate chromosome segregation, it performs three major functions using disparate molecular mechanisms. It operates a mechanosensitive signaling cascade known as the Spindle Assembly Checkpoint (SAC) to detect and signal the lack of attachment to spindle microtubules, and delay anaphase onset in response. After attaching to spindle microtubules, the kinetochore generates the force necessary to move chromosomes. Finally, if the two sister kinetochores on a chromosome are both attached to microtubules emanating from the same spindle pole, they activate another mechanosensitive mechanism to correct the monopolar attachments. All three functions maintain genome stability during cell division. The outlines of the biochemical activities responsible for these functions are now available. How the kinetochore integrates the underlying molecular mechanisms is still being elucidated. In this review, we will discuss how the nanoscale protein organization in the kinetochore, which we refer to as kinetochore ‘architecture’, organizes its biochemical activities to facilitate the realization and integration of emergent mechanisms underlying its three major functions. For this discussion, we will use the relatively simple budding yeast kinetochore as a model, and extrapolate insights gained from this model to elucidate functional roles of the architecture of the much more complex human kinetochore.

Introduction

Multi-protein assemblies and machines assume tremendously diverse composition and organization to perform complex cell biological functions. An excellent example of a protein assembly is the endocytic coat, which is a transient, continuously evolving assemblage of many interacting proteins [1]. At the other extreme, is the nuclear pore. The core scaffold of the nuclear pore is a long-lived structure containing precisely organized copies of many proteins [2]. In both cases, the protein architecture, defined as the nanoscale spatial organization of component proteins within the protein assembly or machine, decides how

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component proteins cooperate with one another to realize their functions. Reductionist methods have been extremely successful in defining structure-function relationships for individual proteins. However, to fully understand multi-protein machines, integrative approaches that define how individual components give rise to emergent functions, and establish 'architecture-function' relationships, are also necessary. The eukaryotic kinetochore presents an excellent case to study architecture-function relationships.

Much is now known about the structures, biochemical activities, and the biophysics of the component proteins of the kinetochore that execute its three major functions (Figure 1A, ref. [3]). However, this knowledge does not fully reveal the underlying molecular mechanisms, explain how the kinetochore integrates these mechanisms into one framework, or predict the possibility of cross-talk among its functions [4]. For this, the spatial organization of the biochemical activities must be considered. Complicating this analysis, however, is the fact that most eukaryotic kinetochores bind multiple microtubules dynamically. For example, the human kinetochore simultaneously interacts with the plus-ends of ~ 20 microtubules that exist as a mixed population of both polymerizing and depolymerizing microtubules. Furthermore, the ~ 200 nm diameter disk-shaped human kinetochore is densely populated with a large and diverse set of proteins, most of which are in multi-copy. In this context, the kinetochore found in the budding yeast *Saccharomyces cerevisiae* is a particularly suitable model, because it stably binds to the plus-end of one microtubule in metaphase [5, 6]. It thus represents the basic functional unit of the eukaryotic kinetochore – one kinetochore-microtubule attachment. Important aspects of the architecture of the yeast kinetochore-microtubule attachment in metaphase have been quantified. Models of kinetochore architecture created from these and structural data provide the starting point needed to study architecture-function relationships [7, 8].

The core protein machinery of the yeast kinetochore is conserved. Therefore, the architecture-function relationships derived from budding yeast will provide insight into the operation of the highly complex human kinetochore. Indeed, a recent study proposed an elegant conceptualization of the human kinetochore as the two-dimensional convolution of multiple yeast kinetochore-like subunits over a disk-shaped surface [9]. Nevertheless, the human kinetochore is built for entirely different performance specifications: it must coordinate the activities of its multiple microtubule binding sites to move the chromosome over longer distances (~ 5 μm versus < 0.5 μm in budding yeast, ref. [10]), against much larger opposing forces (>100 pN versus ~ 7 pN in yeast, refs. [11, 12]). In this review, we will use the budding yeast kinetochore as a starting point for the discussion of architecture-function relationships. We will then highlight how these relationships may fit into the complex architecture of the human kinetochore, and the areas in which the two kinetochores likely diverge.

The composition, assembly pathways, and biochemical activities of the kinetochore

We begin the discussion by briefly describing the essential biochemical activities that execute the three major functions of the kinetochore (please see the recent review by

Musacchio and Desai [3] for a comprehensive discussion of the molecular biology of the kinetochore). We will refer to each protein by the name for the human ortholog followed by a super-scripted name of the corresponding budding yeast protein, if it is different. From the functional perspective, the protein composition and assembly of the kinetochore can be simplified as follows (Figure 1B). The kinetochore interacts with the microtubule and with SAC signaling proteins via a network of three protein complexes: KNL1^{Spc105}, Mis12^{Mtw1}, and Ndc80, collectively referred to as the KMN network [13–15]. This interface is assembled by two parallel pathways initiated by the proteins CENP-C^{Mif2} and CENP-T^{Cnn1} [16–20]. CENP-C^{Mif2} and CENP-T^{Cnn1} are assembled on a well-defined territory on each chromosome, known as the centromere, through their interactions with the centromere-specific histone H3 variant CENP-A^{Cse4} [9, 19, 21]. Although the CENP-C^{Mif2} and CENP-T^{Cnn1} pathways are conserved, their contribution to kinetochore function is species-specific: both pathways are required for the function of the human kinetochore, whereas only the CENP-C^{Mif2} pathway is required in budding yeast [22].

Remarkably, the three mechanisms of end-on microtubule attachment, SAC signaling, and error correction ultimately focus on just two proteins that directly interface the kinetochore with the microtubule and the SAC signaling machinery: Ndc80 and KNL1^{Spc105}. To activate the SAC, the Calponin-Homology (CH) domains of Ndc80, which are globular domains located at the microtubule-binding end of the complex, bind the SAC activator, Mps1 kinase [8, 23–25]. Mps1 phosphorylates conserved motifs within KNL1^{Spc105} to enable these motifs to recruit a number of SAC signaling proteins, and form the Mitotic Checkpoint Complex [26–30]. KNL1^{Spc105} also recruits phosphatases that antagonize Mps1 to facilitate SAC silencing [26]. In addition to the crucial role of recruiting Mps1 for SAC signaling, the CH-domains of Ndc80 also function as the primary binding site that establishes end-on microtubule attachment [31, 32]. To maintain end-on attachments and to generate force, Ndc80 recruits several accessory microtubule-associated proteins (e.g. Dam1 complex in fungi, Ska complex, Astrin/SKAP, etc. in metazoa [33–35]). Finally, the kinetochore destabilizes monopolar attachments by directing the Aurora B^{Ipl1} kinase toward the microtubule-binding domains of Ndc80 and other proteins, thereby weakening their affinity for the microtubule [36–42]. This description of the biochemical activities of kinetochore proteins does not fully explain the underlying molecular mechanisms; knowledge of kinetochore architecture is required to elucidate how these activities cooperate. Furthermore, the functional roles of any reorganization of the kinetochore induced by microtubule attachment or dynamic changes within the architecture during kinetochore movement must also be studied.

The protein architecture in the end-on kinetochore-microtubule attachment

The end-on morphology of the kinetochore-microtubule attachment is highly conserved in all eukaryotes that have been studied to date [6]. Kinetochore researchers recognized early on that this morphology plays an integral role in its functional mechanisms, and proposed generalized models centered on the end-on morphology to explain the functional mechanisms [43–45]. To test the implementation of these model mechanisms, however, it is necessary to first define the biochemical properties and structures of kinetochore components, and then their organization within the end-on kinetochore-microtubule

attachment. The latter part has proven to be a significant challenge. The kinetochore is a network of several protein components, most of which are present in multiple copies. Many of these components contain inherently flexible domains and linkages [3]. Additionally, the microtubule plus-end likely re-organizes this protein network in a functionally significant manner [46, 47]. These issues pose a major obstacle for structural biological approaches in defining its architecture. Resolving the positions of individual molecules in the densely packed kinetochore is also beyond the capabilities of super-resolution microscopy. Therefore, alternative approaches are necessary to determine the architecture.

One such approach is to re-construct kinetochore architecture by answering simpler questions pertaining to its key features (Figure 1C). How many molecules of each protein component does one kinetochore incorporate, and how variable is this number? What is the average position of each component, and are these positions variable? What is the axial and circumferential distribution of protein molecules about their average positions? Quantitative answers to these questions obtained from diverse fluorescence microscopy methods and combined with the known structures of kinetochore proteins established a detailed model of the architecture of the KMN network in the budding yeast kinetochore-microtubule attachment [7, 48–50]. Although this architecture invokes certain assumptions, specifically the circular symmetry of kinetochore proteins around the microtubule diameter and the relative positions of the CH-domains and the Dam1 ring, it has enabled powerful predictions regarding the emergent mechanisms of kinetochore function (discussed in the sections below).

Much work is still needed to synthesize a similar understanding of the architecture of the human kinetochore. The average copy numbers of KMN network molecules per kinetochore, and their organization along the axis of the microtubule in metaphase is known [51, 52]. However, their distribution about the average positions and over the disk-shaped surface of the centromere is unknown, a problem that is significantly complicated by the fact that human kinetochores contain multiple microtubule binding sites (Figure 2A). Identification of the CENP-A^{Cse4} nucleosome as the minimal foundation for assembling the KMN network will simplify this problem to some extent [9, 18]. This finding is useful for proposing a model for the ‘local’ kinetochore architecture, defined as the organization of kinetochore proteins in one kinetochore-microtubule attachment. The bilateral symmetry the CENP-A^{Cse4} nucleosome will impose an orientation and spacing on the two CENP-C^{Mif2} molecules that it recruits (Figure 2B, top). This patterning of CENP-C^{Mif2} will then direct the spatial organization of other centromeric proteins, including CENP-T^{Cnn1}. Thus, the spatial organization of centromeric proteins will ultimately dictate the patterning of KMN network molecules, and hence the architecture of the interface of the human kinetochore with the microtubule plus-end (Fig. 2B, bottom). Beyond the local architecture of KMN molecules within one attachment lies the broader architecture of the kinetochore: the distribution of many such attachments across the disk-shaped surface of the centromere. This broader architecture will influence the ability of the kinetochore to interact simultaneously with many microtubule plus-ends. Defining both the local and broader architecture of the human kinetochore remains a major challenge for the field.

Kinetochore architecture encodes a mechanism for sensing end-on attachment

In most eukaryotes, the kinetochore is unattached at the beginning of mitosis. To avoid chromosome missegregation, it delays cell division by activating the SAC. Typically, the kinetochore first binds laterally to the microtubule lattice, and then converts this interaction into a stable end-on attachment. SAC inactivation occurs once end-on attachments form [23, 53]. Recent work reveals two mechanisms that the kinetochore can use to detect end-on attachment and silence the SAC in response [8, 23, 24]. Both mechanisms rely on the dual role of the CH-domains of Ndc80 as the Mps1 binding site and as the interface for end-on attachment. The first mechanism, studied in human cells, proposes that Mps1 and the microtubule plus-end compete for binding to the CH-domains of Ndc80 [23, 24]. Consequently, end-on attachments displace Mps1 from the kinetochore so that it can no longer phosphorylate $KNL1^{Spc105}$. The second mechanism, which comes from studies in budding yeast, suggests an integral role for kinetochore architecture in implementing the attachment-mediated SAC silencing [8].

The signaling state of the yeast kinetochore is determined by a single change in its architecture that is elicited by end-on attachment. In the unattached, SAC active kinetochore, the CH-domains of Ndc80 are located within 10 nm of the phosphodomain of $KNL1^{Spc105}$ (Fig. 3A, top). Therefore, Mps1 bound to the CH-domains robustly phosphorylates $KNL1^{Spc105}$ and the SAC proteins that it recruits, initiating the SAC. In the attached, SAC inactive kinetochore, the CH-domains and $KNL1^{Spc105}$ phosphodomain are ~ 30 nm apart [50]. This prevents Mps1 from phosphorylating $KNL1^{Spc105}$, thereby disrupting SAC signaling (Fig. 3A, bottom). If the 30 nm gap is experimentally abridged, the yeast kinetochore becomes unable to sense end-on attachment, and the SAC becomes constitutively active [8]. Thus, the yeast kinetochore relies on the separation between Mps1 and its target to detect end-on attachments.

Despite recent progress, two significant questions about the mechanism of SAC silencing remain unresolved. The first question is whether the silencing mechanism exclusively relies on the presence of end-on attachment, or whether it also requires force generation by such this attachment. Recent observations suggest that force generation is not necessary for SAC silencing [54, 55]. This conclusion is consistent with the biochemical competition mechanism for SAC silencing, which does not require any force. It is also consistent with the architecture-based model of SAC silencing: the displacement of two protein domains is unlikely to require a large force [8, 56]. Future biophysical analyses of SAC silencing by the kinetochore will unequivocally establish whether SAC silencing requires significant force generation by end-on attachment. The second question relates to the assumption of the binary, switch-like activation and inactivation of the SAC in the two models. This description is superficially valid for the yeast kinetochore, which can exist in only one of two states: attached or unattached. The binary state description does not apply to the human kinetochore, because it attaches dynamically to ~ 20 microtubule plus-ends that turn over completely in about 4 minutes [57]. Furthermore, study of metaphase kinetochores in Potaroo Kidney (PtK1) cells suggest that the kinetochore possesses ~ 15% excess

microtubule-binding capacity that is unused even in metaphase [58]. To distinguish between partial attachment from a complete lack of attachments, the human kinetochore may use either additional regulation or more complex mechanisms to silence the SAC. For example, to inactivate the SAC, the human kinetochore may use either a temporal threshold defined by a minimum time period that the kinetochore must spend in the attached state, or a number threshold defined by the minimum number of microtubules bound by the kinetochore. Very little is known about the existence or nature of such mechanisms.

The role of kinetochore architecture in driving persistent, bidirectional chromosome movement

As suggested by its name, the major function of the kinetochore is to drive chromosome movement. It produces the force necessary for generating movement by harnessing microtubule polymerization dynamics. It is reasonable to expect that the architecture of the microtubule-binding kinetochore proteins is tailored to suit the changing form and position of tubulin dimers at the plus-end. The kinetochore also recruits motor proteins and microtubule-associated proteins (MAPs) as accessory factors for attachment and force generation. These proteins are expected to occupy positions dictated by their interactions with kinetochore proteins and the microtubule. The relatively simple and well-defined microtubule-binding machinery of the yeast kinetochore and its persistent interaction with one microtubule plus-end in metaphase provides the ideal opportunity to study the significance of its architecture to force generation.

Ndc80 is the linchpin of end-on kinetochore-microtubule attachment (Figure 3B). It uses three microtubule-binding domains: a positively-charged disordered N-terminal tail of the Ndc80 subunit and the CH-domains of the Ndc80 and Nuf2 subunits [59]. The disordered tail binds to the negatively-charged tubulin tails. This binding assists in the initial contact between the kinetochore and the microtubule lattice [60]. The CH-domains bind in the groove between tubulin monomers along a straight tubulin protofilament in the microtubule lattice, but they cannot do so if this groove is distorted, as in a curling, depolymerizing protofilament [32, 61]. This property of the CH-domains is essential for forming end-on kinetochore-microtubule attachments [13, 62]. Ndc80 structure appears to be tailored for both lateral and end-on attachment: it contains a flexible hinge in its front section to enable the CH-domains access to the binding groove between tubulin monomers by making a 40° angle to the microtubule axis [63]. The conformation of Ndc80 in metaphase yeast kinetochores suggests that they can assume the preferred orientation for the CH-domains to bind to the lattice (Figure 3B, top, [7]).

Ndc80 positions the kinetochore at the plus-end, but it cannot hold on to a dynamic plus-end against high opposing forces [64]. In budding yeast, the Dam1 complex, which is recruited by Ndc80, is essential for force generation [38, 65]. Dam1 molecules likely assemble in the form of an oligomeric ring encircling the microtubule [7, 33, 66]. The Dam1 ring mechanically opposes the outward curling of tubulin protofilaments during depolymerization, and thus, experiences a poleward force [67, 68]. However, to generate force in this manner, the Dam1 ring must be positioned at the edge of the microtubule lattice

where it can encounter curling protofilaments [69]. Therefore, it is not surprising that Dam1 localizes in close proximity to the CH-domains of Ndc80, which likely bind to the microtubule lattice near the plus-end (Figure 3B, top [7, 50]). It is reasonable to expect that the Dam1 ring is positioned on the centromeric side of the CH-domains so that it can transmit the force generated to the centromere via Ndc80 [7]. The mechanical opposition to microtubule depolymerization offered by the Dam1 complex also institutes a crucial regulatory mechanism known as 'tension-dependent rescue' of the depolymerizing plus-end [11, 38, 70]. As the opposing pull of sister kinetochores on the centromere increases, the Dam1 ring inhibits the conformational change that the tubulin dimers undergo to depolymerize. This force-dependent inhibition promotes the transition from microtubule depolymerization to polymerization. Consequently, the kinetochore switches its direction of movement, and relieves centromeric tension in the process. Tension-dependent rescue is necessary for persistent attachment of sister kinetochores to spindle microtubules.

The high combined affinity of the Ndc80-Dam1 ring assembly likely makes its diffusion along the microtubule lattice very slow [67]. If diffusion is much slower than the rate of microtubule polymerization, then biased-diffusion may not be able keep pace with the polymerizing microtubule tip. The higher affinity of the Dam1 complex for GTP-tubulin, which is present only at the growing microtubule tip, over GDP-tubulin present in the lattice enables Dam1 monomers and small oligomers to track growing microtubule tips [38, 71]. However, whether Dam1 rings can also do this is unclear. Indeed, yeast kinetochore particles have not been observed to track polymerizing microtubule plus-ends *in vitro*, unless they are experimentally assisted by the imposition of an external force directed toward the plus-end [11]. Centromeric strain will drag the kinetochore toward the plus-end *in vivo*, but this process depends on the magnitude of the strain, and it is independent of the growth of the plus-end (Figure 3B, middle). This means that the movement induced by high centromeric tension could potentially slide the kinetochore off the polymerizing microtubule tip, and additional mechanisms may be necessary to mitigate this possibility. In this context, the involvement of $XMAP215^{Stu2}$ in yeast kinetochore motility is noteworthy, but also perplexing. Although $XMAP215^{Stu2}$ is a well-known tubulin polymerase, it destabilizes microtubules during mitosis in yeast cells [72, 73]. Consistent with this finding, $XMAP215^{Stu2}$ localizes in a region within the yeast kinetochore where the polymerizing plus-end is expected to be located (Figure 3B, middle) [7]. On the other hand, *in vitro* findings suggest that $XMAP215^{Stu2}$ stabilizes attachment of yeast kinetochores to the microtubule tip in a tension-dependent manner [74]. We suggest a simple model to reconcile these observations (Figure 3B, middle & bottom panels). We propose that $XMAP215^{Stu2}$ recognizes polymerizing microtubule plus-ends within yeast kinetochores and promotes their transition to depolymerization. This prevents the plus-ends from outpacing the kinetochore, and minimizes the possibility of the kinetochore sliding off a growing microtubule tip under high centromeric tension.

The conserved mechanics and biochemistry underlying microtubule plus-end dynamics suggest that the basic mechanisms of harnessing force and persistent bidirectional movement used by the budding yeast kinetochore will be conserved in human kinetochores. This is reflected in the similar organization of microtubule-binding activities in the yeast and human kinetochore [50, 52]. Ndc80 is similarly organized and required for end-on attachment in

both kinetochores [50, 52, 75]. However, the human kinetochore attaches to ~ 20 microtubule plus-ends on average, and these attachments are dynamic: they turn over with a half-life of ~ 4 minutes (in RPE-1 cells [76] compared to ~ 25–30 minutes duration of mitosis [10]). The human kinetochore also employs a much larger array of accessory motors and MAPs to elaborate on the basic mechanisms of force generation. The motors include MCAK, Kif18A, Dynein, and CENP-E [77–80]. The MAPs include the Ska complex, Astrin/SKAP, CLASP, EB1, and XMAP215^{Stu2} [34, 35, 81–84]. The mechanisms of kinetochore recruitment and organization of some of these accessory proteins are similar to the corresponding mechanisms in yeast [52, 77–80, 85]. For example, the recruitment and location of the Ska complex in the human kinetochore appears similar to that of Dam1 in the yeast kinetochore [85]. The collective activities of these proteins in microtubule-binding and in modulating plus-end polymerization dynamics establish and maintain end-on kinetochore-microtubule attachment, generate force, and coordinate the activities of sister kinetochores to drive persistent, bidirectional chromosome movement.

One puzzling aspect of the large array of accessory motors and MAPs employed by the human kinetochore is the apparent redundancy in their activities and kinetochore-specific functions. For example, Ska and XMAP215 are involved in microtubule attachment and force generation; EB1, CLASP, and Astrin/SKAP promote plus-end polymerization [34, 74, 84, 86, 87]. This redundancy may be necessary in part to achieve robust kinetochore functionality. However, it is also possible that the unique kinetochore position of some of these proteins ascribes unique functions to these proteins. For example, MCAK and Kif18A can both destabilize the plus-end [77, 88]. However, MCAK improves the coordinated movement of sister kinetochores, whereas Kif18A promotes the mutually antagonistic activity of the sisters and reduces their coordinated movements [77, 78]. In addition to differences in the biochemical activities of these motors, how they encounter the microtubule plus-end in the kinetochore may give rise to the differences in their function. MCAK localizes at the centromere, whereas Kif18A walks along the microtubule to reach the plus-ends (Figure 2A–B, [89]). Due to its centromeric localization, MCAK may selectively destabilize only those plus-ends that polymerize to extend more than usual towards the centromere. In contrast, Kif18A-mediated plus-end destabilization may be dependent on microtubule length/age [78, 90]. Thus, differences in function may arise from differences in protein position. Therefore, to fully understand how the human kinetochore brings about bidirectional chromosome movement, the biochemical activities as well as the nanoscale architecture of its microtubule-binding machinery must be studied. Additionally, any temporal coordination of these activities and dynamics of protein architecture are also likely to play key roles in kinetochore motility [91]. A major challenge in the field is to formulate a comprehensive model that explains how the human kinetochore synthesizes the activities of its microtubule-binding proteins to control plus-end dynamics, and generate persistent, bidirectional chromosome movement.

Potential roles of kinetochore architecture in correcting monopolar attachments

Although bipolar attachment of sister kinetochores to microtubules emanating from opposite spindle poles is strongly favored, two types of erroneous attachments occur frequently: merotelic and monopolar [57, 92, 93]. The first type of erroneous attachments is known as merotelic attachment, wherein a single kinetochore attaches to microtubules emanating from both poles. There is no kinetochore-based mechanism for the resolution of merotelic attachments; these attachments are destabilized, and bipolar attachments stabilized, by a finely tuned regulation of the dynamicity of spindle microtubules [57, 94]. The second type of erroneous attachments are known as syntelic or monopolar attachments, wherein both sister kinetochores in a pair are attached to the same pole. These attachments are destabilized by a dedicated, kinetochore-based error correction process. Two elements of this process are clear. First, it directs Aurora B^{Ipl1} kinase activity toward microtubule-binding kinetochore proteins to weaken their microtubule-binding affinity [36–42, 95]. Second, the activity of the error correction process is strongly correlated with a sustained lack of centromeric tension, which is a characteristic unique to sister kinetochores with monopolar attachments [36]. Interestingly, sister kinetochores with bipolar attachments also experience periodic and transient loss of centromeric tension, but they do not activate the error correction process. How the kinetochore senses a prolonged absence of centromeric tension, and activates Aurora B^{Ipl1} in response, is unclear. Potential mechanisms that can explain this error correction process have been discussed in excellent reviews (e.g. see [92, 96]). Therefore, we will only describe the prevalent model, and focus on the potential role of kinetochore architecture in the error correction process.

The prevalent model postulates that centromeric tension in the human kinetochore separates Aurora B^{Ipl1} from its phosphorylation targets in a manner that is superficially similar to the mechanism of attachment-dependent SAC signaling [36, 97]. Aurora B^{Ipl1} dynamically localizes to chromatin situated in between the sister centromeres (Figure 2A). Here, the high local concentration of Aurora B^{Ipl1} stimulates its auto phosphorylation and hence hyper-activation. Super-imposition of a phosphatase activity on this Aurora B^{Ipl1} hyper-activation region creates a steep gradient in Aurora B^{Ipl1} kinase activity (Figure 3C) [97]. Sister kinetochores with monopolar attachment fall within the region of Aurora B^{Ipl1} hyperactivity, whereas kinetochores with bipolar attachment deform the centromere and emerge out of it. Consequently, microtubule-binding proteins in these kinetochores become dephosphorylated, and microtubule attachment is stabilized. Although this elegant mechanism is strongly supported by data from vertebrate kinetochores, it does not explain a key observation from budding yeast. Budding yeast kinetochores correct monopolar attachments even when Aurora B^{Ipl1} is unable to localize to the centromere [98]. Therefore, additional mechanisms in the error correction process remain to be discovered in both yeast and humans.

Kinetochore architecture is clearly important in the prevalent model of the error correction process, because it determines the positions of Aurora B^{Ipl1} targets relative to the centromere-localized Aurora B^{Ipl1}. However, the architecture of kinetochores with

monopolar and bipolar attachments is likely to be similar given that they both possess end-on attachment. A key difference in kinetochore architecture under the two scenarios may be that the plus-end is in close proximity to the centromere only in kinetochores with monopolar attachment (Figure 3C). One possibility is that the error correction mechanism uses the proximity between centromere-localized Aurora B^{Ipl1} and the microtubule plus-end to locally concentrate, and then effectively transport active Aurora B^{Ipl1} along the microtubule to microtubule-bound kinetochore proteins [98]. However, the role of kinetochore architecture, if any, in mechanisms that direct Aurora B^{Ipl1} activity selectively to kinetochores with monopolar attachments remains poorly understood.

Concluding remarks

The nanoscale architecture of the kinetochore can provide insight into how it integrates three truly disparate mechanisms in one molecular framework. Comparison of the yeast and human kinetochores also reveals how the basic integration of the three mechanisms may be enhanced to meet species-specific functional requirements. In the integrative model of the eukaryotic kinetochore, Ndc80 emerges as the focal point of all three mechanisms. It acts as the terminal of a switch that controls the SAC, the conformational sensor that positions the kinetochore at the plus-end, an organizer of microtubule-binding activities in the kinetochore, and a major target of phosphoregulation by Aurora B^{Ipl1}. Therefore, the spatial patterning of Ndc80 in the kinetochore and its architecture relative to the microtubule plus-end will play key roles in shaping the emergent mechanisms underlying all three kinetochore functions. Future studies of the kinetochore that explicitly test the role of kinetochore architecture in its functional and regulatory mechanisms will lead us to a comprehensive understanding of one of the most fascinating multi-protein machines in cell biology.

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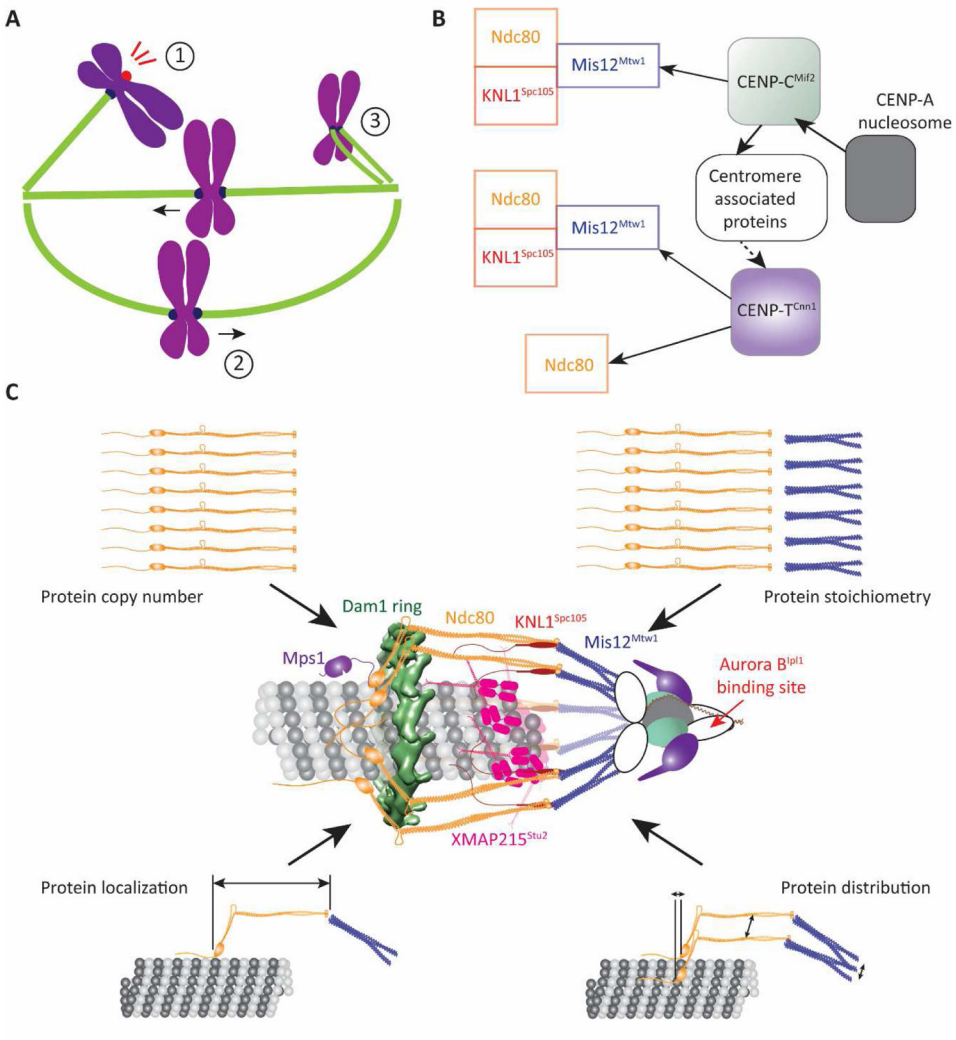

Figure 1. The function and protein architecture of the kinetochore

A Cartoon of a mitotic spindle displaying the three main kinetochore functions: (1) Activation of the Spindle Assembly Checkpoint, (2) generation of bidirectional chromosome movement that is coupled to microtubule polymerization and depolymerization, and (3) correction of monopolar attachment of sister kinetochores. **B** (left to right = microtubule plus-end to centromere) The conserved, dual pathways (solid arrows – direct interaction, dashed arrow – indirect interaction) that assemble the KMN network, which forms the interface of the kinetochore with the microtubule plus-end. **C** Reconstruction of the protein architecture of the budding yeast kinetochore using fluorescence microscopy measurements and protein structures [7, 8, 14, 15, 32, 33, 48–50, 73]. Centromere-associated proteins are represented by white, oblong shapes.

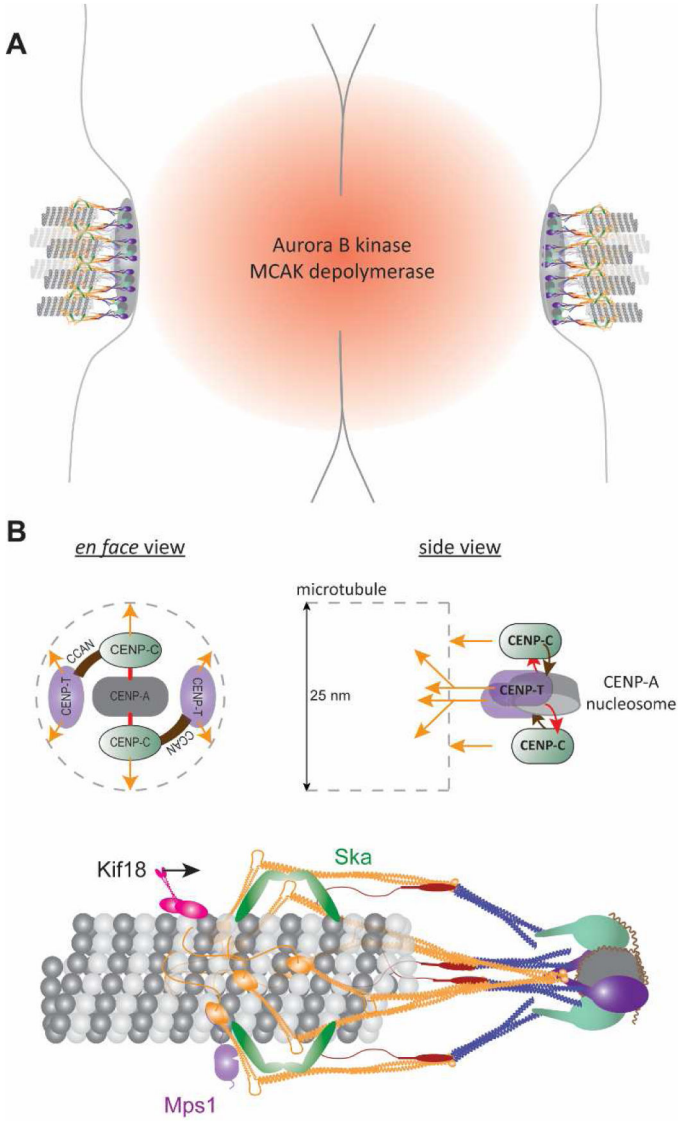

Figure 2. Protein architecture of the human kinetochore

A Cartoon of the organization of sister kinetochores on a human chromosome. The inter-centromeric localization of Aurora BIpl1 and MCAK is highlighted. **B** Top: Schematic displays a hypothetical spatial manifestation of the biochemical pathways of kinetochore assembly. Orange arrows indicate pathways of Ndc80 recruitment; grey dashed lines represent the microtubule. Note that CENP-T^{Cnn1} recruits two Ndc80 molecules. Bottom: Cartoon of a hypothetical local architecture of the kinetochore-microtubule attachment in humans.

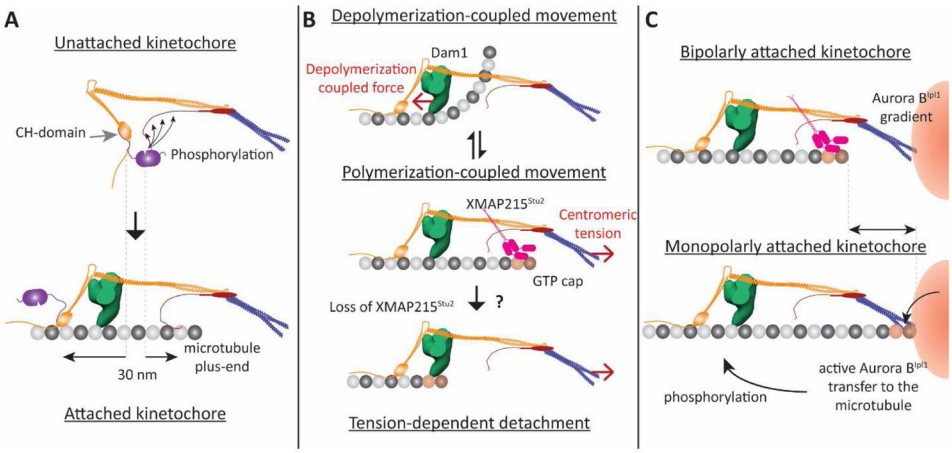

Figure 3. Proposed architecture-function relationships for the yeast kinetochore (1-D representations of the kinetochore shown)

A Role of kinetochore architecture in SAC inactivation: Separation of the CH-domains of Ndc80 and the phosphodomain of KNL1^{Spc105} by end-on attachment (highlighted by dashed lines) disrupts the phosphorylation of KNL1^{Spc105} by the Mps1 kinase (magenta) bound to the CH-domain. **B** Proposed roles of the architecture of microtubule-binding proteins in generating bidirectional movement. Top: When the plus-end is depolymerizing, the Dam1 ring (green) mechanically opposes the curling of tubulin protofilaments, and experiences a pushing force (red arrow). Middle panel: We propose that XMAP215^{Stu2} localizes to the kinetochore by recognizing the GTP-tubulin cap on the polymerizing plus-end. Its microtubule-destabilizing activity reverts the plus-end back to the depolymerizing state. Bottom panel: In the absence of XMAP215 activity, centromeric tension can slide the kinetochore off the growing plus-end. **C** Potential roles for kinetochore architecture in correcting monopolar attachment: The position of the plus-end may be significantly different in kinetochores with bipolar and monopolar attachment (highlighted by the arrow, top and bottom panels respectively). The proximity of the lattice to the centromere may facilitate the transport of hyper-activated Aurora B^{Ipl1} kinase to its targets – microtubule-binding kinetochore proteins.