Kinetochore function: **The complications of becoming attached**

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Kinetochores are specialized protein complexes assembled on centromeric DNA that connect to spindle microtubules and mediate proper chromosome segregation during cell division. Recent results have implicated specific kinetochore proteins in microtubule attachment.

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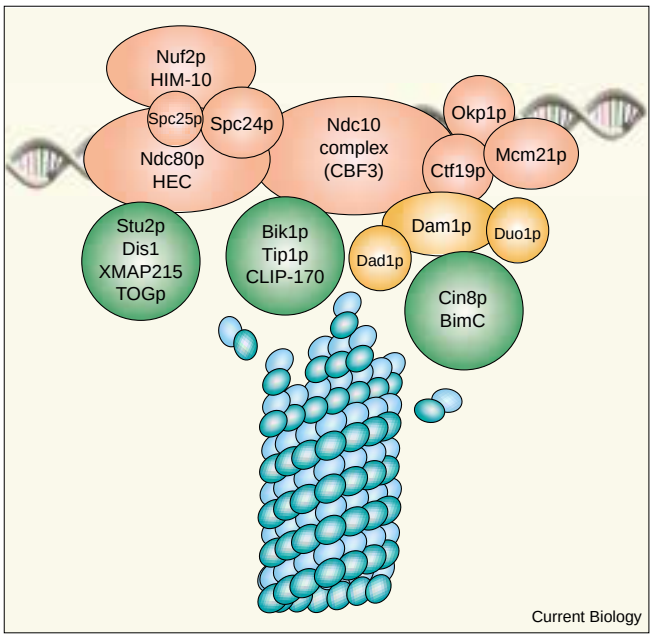
Our understanding of kinetochores has progressed significantly since the first descriptions of these cellular structures based on electron microscopic observations [1]. Spindle microtubules connect end-on to chromosomes specifically at kinetochores, predominately at the outer kinetochore plate and to a lesser extent to the underlying chromatin [2,3]. Upon attachment, kinetochores move chromosomes on the spindle, generating tension. In the absence of proper chromosome attachment and spindle tension, checkpoint mechanisms prevent progression through mitosis [4]. While the attachment of DNA to microtubules is clearly a fundamental role of kinetochores, we are only beginning to elucidate the specific mechanisms involved. Recent studies in the budding yeast *Saccharomyces cerevisiae* have revealed a number of conserved factors required for this process (Figure 1), adding to an already sizable contingent of known kinetochore proteins in this organism.

Figure 1



A diagram illustrating the components of the budding yeast kinetochore that have been implicated in microtubule attachment. The names of homologous proteins in different organisms are also given. Overlap of components denotes biochemical and/or genetic interactions. The effects of mutation or absence of a component is coded by color: red component defects cause a complete loss of chromosome attachment to the spindle; orange component defects cause monopolar attachment or loss of bipolar attachment phenotypes; and green component defects have a less severe effect, characterized by mitotic delay and/or diminished chromosome movement.

The awesome power of yeast cytology

Although budding yeast has provided an invaluable system for genetic identification of proteins required for accurate transmission of chromosomes, it has not been widely acclaimed for informative cytology. However, recent work using live imaging of fluorescently tagged centromeres in budding yeast has provided a microscopy-based approach to evaluating centromere behavior. These studies have revealed that kinetochores are found in two separate regions between the spindle center and each pole, a distribution thought to result from transient separation of sister centromeres [5,6].

He *et al.* [7] capitalized on this distinctive behavior and systematically examined the localization of candidate proteins tagged with green fluorescent protein (GFP) for a bilobed distribution on the spindle, and evaluated the effects of their disruption on sister centromere dynamics. Kinetochore localization of all of the proteins was further confirmed by chromosome immunoprecipitation (ChIP), in which immunoprecipitated protein is subject to analysis by the polymerase chain reaction (PCR) with centromere-specific primers. The analyses provided support for recent genetic and biochemical evidence that the Ndc80 complex, containing proteins Ndc80p, Nuf2p, Spc24p and Spc25p, is critical for kinetochore function [8,9]. The results also suggest potential kinetochore roles for three known microtubule-associated proteins (MAPs), Dam1p, Stu2p and Bik1p, as well as a microtubule-based motor protein, Cin8p.

The Ndc80 complex is required for kinetochore assembly

Defects in any component of the Ndc80 complex disrupt centromere dynamics by causing complete loss of chromosome attachment to the spindle [7,9]. Association of the Ndc80 complex with kinetochores is dependent on another complex, CBF3, which is composed of Ndc10p, Cep3p, Ctf13p and Skp1p. CBF3 interacts directly with centromeric

DNA, forming the core structure required to recruit the Ndc80 complex and other factors, including the three subunit Ctf19 complex containing Ctf19p, Mcm21p and Okp1p [10,11].

Unlike CBF3, the Ndc80 complex is conserved in other organisms. Homologues of Ndc80p, Nuf2p and Spc24p have been localized to centromeres in the fission yeast *Schizosaccharomyces pombe* [8,10]. Centromere localization of the human homologues of Ndc80p and Nuf2p has also been reported [8,12]. Microinjection of cells with antibodies against the human homologue of Ndc80p, called HEC, resulted in a failure of chromosomes to coalesce at the metaphase plate and segregate properly prior to cytokinesis [12]. Further study of Ndc80p (HEC) and other components of the complex in animal cells is of primary interest.

Three different experimental approaches suggest that the Ndc80 complex directly contributes to kinetochore assembly. First, the morphology of kinetochores has been examined by electron microscopy. This is not possible in *S. cerevisiae*, where kinetochore ultrastructure is not apparent, but recent studies of the Nuf2p homologue, HIM-10, in the nematode *Caenorhabditis elegans* support a role for the Ndc80 complex in kinetochore assembly. Like monocentric animal centromeres, the holocentric centromeres of this organism are thought to underlie repetitive kinetochore substructures that appear similar in morphology by electron microscopy [3,13,14]. HIM-10 disruption in embryos by RNAi resulted in lagging and mono-oriented chromosomes and aberrant kinetochore morphology, with the outer coronal layer absent [13].

A second gauge for specific disruption of microtubule-kinetochore attachment is the integrity of the spindle-assembly checkpoint, which depends on proper kinetochore structure [4,15]. Defects in kinetochore factors can compromise checkpoint function as severely as defects in the checkpoint components themselves. Mutations in *NDC10*, *NDC80* and *SPC24* were found to severely disrupt kinetochore assembly and abolish checkpoint integrity [8,16]. In contrast, mutations in *DAM1* or *STU2*, for example, were found to cause irregular microtubule-kinetochore attachments, but the mutant cells still arrest in a checkpoint-dependent fashion [17,18].

Third, yeast mutants lacking Ndc80 have been tested for defects in localization of other kinetochore components. For example, when Ndc80p or Spc24p are defective, many kinetochore components appear to assemble normally, including Ctf13p, Ctf19p, Mcm21p, Okp1p and Spc24p [7,8]. This contrasts with cells harboring mutations affecting the CBF3 complex, which fail to target most known kinetochore components [5,7,11]. Further analysis of this kind will be required to understand how the many kinetochore components assemble. Altogether, these data suggest that the Ndc80 complex is important for kinetochore assembly and forms a link between core structures and microtubule-binding components of the kinetochore.

Exploring the outer layers

Spindle-proximal components of the kinetochore are also complicated. Dam1p, Stu2p, Bik1p and Cin8p all display a broader localization pattern on the spindle than the Ndc80 complex [7,17,18]. As more spindle proteins are analyzed by fluorescence microscopy and ChIP, we will gain a better understanding of the specificity of kinetochore localization by these techniques. Dam1p and Stu2p may also play roles in spindle function, and their mutant phenotypes could result at least partially from loss of spindle integrity as well as defects in microtubule-kinetochore interaction.

On the basis of genetic, biochemical and phenotypic data, Dam1p is likely to function specifically in kinetochore-microtubule attachment. Dam1p binds directly to microtubules and is part of a protein complex containing Duo1p and Dad1p which associates specifically with kinetochores in chromosome spreads [17]. *Dam1* alleles interact genetically with genes encoding the kinetochore proteins Ctf19p and Bir1p, as well as Stu1p and Cin8p [17,19]. Kinetochore-specific roles are indicated, as certain mutations of Dam1p lead to monopolar attachment of chromosomes to the spindle [7,17]. Careful examination of conditional mutants suggests that Dam1p is required to form, but not to maintain, kinetochore–spindle attachment [17].

Disruption of Stu2p, like that of Dam1p, does not completely abolish kinetochore–spindle interactions, but it does compromise kinetochore positioning and transient sister separation [7]. Requiring both Ndc10p and Ndc80p for kinetochore localization, Stu2p is also found at cortical capture sites along the periphery of cells, suggesting that it may preferentially associate with the plus ends of microtubules [7]. In *S. pombe*, the Stu2p homologue Dis1p associates with the centromere and is required for correct chromosome movement and transient sister separation [20,21]. Defining a direct role for Stu2p in kinetochore function is complicated, however, by its central role in microtubule stabilization, which is required for spindle elongation during anaphase [18]. This spindle stabilizing function is conserved among eukaryotes, as depleting the *Xenopus* homologue XMAP215 from mitotic egg extracts results in a shorter steady-state length of microtubules and consequently small, distorted spindles [22].

Perhaps because of their functional redundancy with other factors, Bik1p and Cin8p are both non-essential proteins, dispensable for generating spindle tension and chromosome movements [7]. Bik1p localizes to kinetochores in an Ndc10p-dependent manner, as well as to spindle

microtubules and cortical attachment sites [7]. The human Bik1p homologue, CLIP-170, localizes to kinetochores and selectively binds microtubule plus ends throughout the cell [23,24]. Overexpression of a mutant CLIP-170 lacking the microtubule-interaction domain displaced endogenous CLIP-170 at the kinetochore and delayed prometaphase, but chromosomes ultimately attached and progressed through mitosis, suggesting a supporting role for this protein in kinetochore attachment [24].

The observation that the motor Cin8p localizes to yeast kinetochores is intriguing. Metazoans have been shown to possess a number of kinetochore motors that participate in chromosome attachment or motility on the spindle, including the plus-end directed motor CENP-E, the minus end-directed motor dynein and the microtubule-destabilizing motor MCAK/XKCM1 [25]. In contrast, although budding yeast motors participate in anaphase chromosome segregation [26], they have not yet been shown to have kinetochore-specific roles. Cin8p is most similar to the ubiquitous BimC class of spindle motors, which are thought to maintain spindle pole separation. It will also be of great interest to evaluate a potential role for the Kip3p motor, as it shares some sequence and functional homology with MCAK/XKCM1 [18]. As yeast motors often have overlapping functions, careful analysis of kinetochore behavior in cells lacking multiple motors will be required to elucidate their roles.

Conclusions: getting the most out of your MAPs

Microtubule-binding proteins function at the kinetochore to mediate at least three vital activities: chromosome attachment to the spindle, coordination with the spindle assembly checkpoint, and poleward chromosome movement during anaphase. At least 30 proteins have already been identified to function at the kinetochore in budding yeast, but those involved in attachment to the spindle are just beginning to be elucidated. As in the spindle, MAPs and motors likely play multiple and partially overlapping roles at the kinetochore.

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