Cell

Microtubule Capture: A Concerted Effort

Chitra Kotwaliwale[1] and Sue Biggins[1,*]
[1]Fred Hutchinson Cancer Research Center, Division of Basic Sciences, 1100 Fairview Avenue North, A2-168, Seattle, WA 98109, USA
*Contact: sbiggins@fhcrc.org
DOI 10.1016/j.cell.2006.11.032

Kinetochores direct attachment of chromosomes to microtubules of the mitotic spindle during cell division. Three recent studies in *Cell*, including one in this issue, reveal important new roles for two kinetochore protein complexes—Ndc80 and INCENP-Survivin—in establishing the correct attachment of chromosomes to spindle microtubules (Cheeseman et al., 2006; DeLuca et al., 2006; Sandall et al., 2006).

Introduction

Accurate chromosome segregation is critical to prevent aneuploidy, a condition associated with tumorigenesis and a number of birth defects. Chromosome segregation depends on the interaction between chromosomes and spindle microtubules, dynamic polymers of repeating α/β tubulin dimers. Microtubules possess an inherent polarity: their minus ends are always proximal to the spindle pole and their dynamic plus ends are distal to the spindle pole and interact with chromosomes. The connection between the microtubules and chromosomes is mediated by the kinetochore, a multiprotein complex that assembles on centromeric DNA (reviewed in Cleveland et al., 2003). A prerequisite for anaphase onset is that each pair of replicated sister kinetochores is bioriented, that is, attached to microtubules emanating from opposite spindle poles. However, the process of biorientation is prone to errors and often results in inappropriate kinetochore-microtubule interactions that must be detected and eliminated.

Despite the identification of more than 60 kinetochore proteins, the specific kinetochore components that directly mediate attachment to microtubules and the molecular mechanism by which cells detect and correct inappropriate kinetochore-microtubule interactions remain unknown. Three recent studies in *Cell* (Cheeseman et al., 2006; DeLuca et al., 2006; Sandall et al., 2006) shed light on these questions.

The Regulation of Chromosome Attachment to Microtubules

During every cell cycle, a variety of incorrect kinetochore-microtubule configurations can occur. Monotelic attachments arise when only one sister kinetochore binds to microtubules, syntelic attachments result when both sister kinetochores bind to microtubules from the same pole, and merotelic attachments occur when one or both sister kinetochores bind to microtubules from both poles. Although monotelic attachments can be detected based on the absence of microtubule binding at one kinetochore, syntelic and merotelic attachments pose an intriguing problem because they cannot be distinguished based on the simple presence or absence of microtubule binding. Pioneering studies performed more than 30 years ago implicated mechanical tension as a key signal used by the cell to monitor kinetochore-microtubule attachments (reviewed in Pinsky and Biggins, 2005). Sister kinetochores come under tension when they biorient because the pulling forces exerted by microtubules from opposite poles are opposed by the linkage between sister chromatids. Kinetochore-microtubule arrangements that generate normal tension are selectively stabilized, whereas those that fail to generate the proper amount of tension are unstable.

To date, the only protein identified that appears to detect inappropriate attachments based on tension at kinetochores is the Ipl1/Aurora B protein kinase. Aurora B forms a complex called the chromosomal passenger complex that contains the inner centromere protein (INCENP), Survivin, Dasra B/Borealin/Csc1, and Dasra A (reviewed in Vagnarelli and Earnshaw, 2004). Moreover, INCENP is a potent activator of Aurora B kinase activity. Studies in budding yeast and cell culture have suggested that Aurora B specifically destabilizes kinetochore microtubules that are syntelically or merotically attached (reviewed in Pinsky and Biggins, 2005). However, the molecular mechanism by which Aurora B detects tension and promotes the instability of inappropriate microtubule attachments has remained elusive, in part because the kinetochore proteins that directly mediate microtubule binding are still unknown.

What Is the Core Microtubule Attachment Site at the Kinetochore?

Although the loss of function of many kinetochore proteins leads to defects in microtubule binding, it has been difficult to directly implicate any of these factors in mediating core attachment at the kinetochore. Extensive work has clearly shown that although most kinetochore mutants contain chromosomes that are not attached to microtubules, it appears to be a secondary consequence of the Ipl1/Aurora kinase-mediated correction mechanism that destabilizes defective microtubule attachments (Dewar et al., 2004; Lampson and Kapoor,

2005; Pinsky et al., 2006). Improper microtubule attachments are therefore maintained in most kinetochore mutants when Aurora B activity is absent. This suggests that either none of the proteins tested are required for microtubule binding or that there are multiple microtubule-binding components at the kinetochore. Two recent papers have made significant progress toward identifying key kinetochore components that constitute core attachment activity.

The conserved Ndc80 complex is an excellent candidate to mediate core microtubule attachment because it localizes to the outer plate of the vertebrate kinetochore, the region of the kinetochore where microtubule plus ends terminate (DeLuca et al., 2005). DeLuca et al. (2006) analyzed the role of the Ndc80/Hec1 complex in microtubule attachment. In contrast to the depletion of Ndc80 by small interfering RNA that resulted in unstable kinetochore microtubule interactions, the investigators found that microinjection of an antibody that specifically blocks the N-terminal globular domain of Ndc80 in mitotic PtK1 cells led to robust microtubule-kinetochore attachments. However, there was a significant increase in merotelic attachments, a phenotype reminiscent of Aurora B downregulation. In addition, kinetochore pairs in antibody-injected cells failed to show normal oscillatory movements, indicating that microtubule plus-end dynamics were affected. Because Aurora B likely releases microtubules via phosphorylation of one or more core microtubule-binding components of the kinetochore, a failure to be phosphorylated by Aurora B should result in stable but incorrect kinetochore-microtubule interactions. The phenotypes produced by the antibody led DeLuca et al. (2006) to propose that the N terminus of Ndc80 contains key Aurora B phosphorylation sites that cause microtubule detachment when phosphorylated. By blocking access to these phosphorylation sites, the antibody would lead to aberrant attachments. Consistent with this, they found that Aurora B phosphorylates the N terminus of Ndc80 in vitro and elimination of these sites resulted in chromosome alignment defects and an increase in merotelic attachments. The authors therefore proposed that Ndc80 links the kinetochore to microtubules directly or via another factor. In response to inappropriate attachments, Aurora B phosphorylation of either Ndc80 or another factor would reduce their affinity for microtubule binding, thereby detaching microtubules from kinetochores.

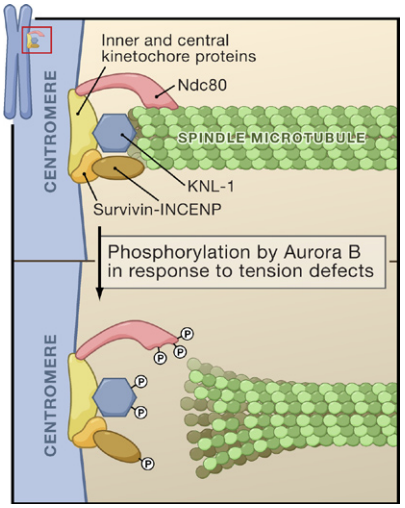

Figure 1. Kinetochore Attachment to Microtubules and Its Regulation by Aurora B

The kinetochore—a proteinaceous structure that forms on centromeric DNA—is composed of multiple low-affinity microtubule-binding components. Although the Ndc80 complex binds to the microtubule lattice, other components may bind to the microtubule plus end. In response to tension defects, Aurora B phosphorylates the microtubule-binding proteins to decrease the affinity of these factors for microtubules. In turn, this leads to detachment of microtubules from kinetochores. Factors such as INCENP-Survivin may perform multiple functions at the kinetochore. In addition to serving as tension sensors that are regulated by Aurora B, these molecules may also promote core microtubule-binding activity.

It is tempting to think that the Ndc80 complex may be the core microtubule-binding component of the kinetochore. In that case, cells lacking Ndc80 would exhibit unattached kinetochores even in the absence of Aurora B activity. However, when Aurora B is impaired in budding yeast *ndc80* mutant cells, the defective kinetochores are able to bind to microtubules weakly (Pinsky et al., 2006). Therefore, additional factors likely act in parallel to Ndc80 to promote microtubule binding.

Another group of investigators identified additional core microtubule-binding activity biochemically. Cheeseman and colleagues analyzed the biochemical properties of the *Caenorhabditis elegans* KMN network that contains *K*NL-1, the *M*is12 complex, and the *N*dc80 complex and is required for normal kinetochore function (Cheeseman et al., 2004, 2006). Using the elegant approach previously used to reconstitute the budding yeast DAM1/DASH complex and demonstrate that it forms rings around microtubules (Miranda et al., 2005; Westermann et al., 2005), the authors successfully reconstituted each subcomplex in bacteria by coexpressing the open reading frames of each complex from individual plasmids (Tan, 2001). Using gel filtration, the authors showed that the Ndc80 complex does not independently interact with either KNL-1 or the Mis12 complex and the KMN network can only be fully reconstituted when all three constituents are present. The investigators next analyzed the microtubule-binding activities within the KMN network. Although the Mis12 complex did not interact with microtubules, KNL-1 and the Ndc80 subcomplex bound microtubules independently. However, careful measurements of binding affinities showed that these interactions were quite weak. Strikingly, the reconstituted KMN complex resulted in a synergistic increase in the microtubule-binding capacity of the network. Based on these in vitro microtubule assays, Cheeseman et al. (2006) proposed that the kinetochore microtubule interface is likely composed of an array of low-affinity binding sites comprised of KMN and other factors that cooperate to create a dynamic kinetochore-spindle interface (Figure 1). Consistent with this, the authors performed a beautiful ultrastructural analysis of the purified Ndc80 complex and found that it bound along the length of the microtubule lattice at a specific angle. The binding of the