be visualized as the N-MBDs acting as a mechanical brake of the transporting machinery by physically restricting the rate-limiting movement.

The described model certainly leaves several structural questions unanswered. How, or where, does the Cu^{+}-loaded chaperone interact with the ATPase? Can the arrangement of transmembrane segments be better defined? How are multiple N-MBDs accommodated in the structure? Interestingly, these issues are within the reach of cryo-EM approaches. As in the case of the role of N-MBDs, addressing these would have a significant impact in the field.

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Insights into the Kinetochore

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The Ndc80 complex is a core component of the kinetochore, which links chromosomes to microtubules. Recently, Ciferri et al. (2008) published an atomic-level structure of the complex with implications for kinetochore architecture and for the generation and control of chromosome movements during mitosis.

Background

All life depends on the accurate distribution of duplicated DNA during cell division. In eukaryotic cells, this process is carried out by an integrated molecular machine, the mitotic spindle, named in the 1800s for its similarity to a part of the spinning wheel from that time. Beyond its shape, however, the mitotic spindle bears little resemblance to its yarn-spinning namesake. It has four key components, each a fascinating molecular machine in its own right (Figure 1A): (1) the replicated chromosomes, or sister chromatids, which are held together in pairs until the spindle is fully assembled; (2) the spindle poles, which organize the microtubules; (3) the microtubule fibers, which extend from the spindle poles; and (4) the kinetochores, specialized structures on each chromosome where the microtubules attach.

Kinetochores form a bridge between the chromosomes and the microtubule fibers, and they are at the nexus of the mitotic process (for review, see Cheeseman and Desai, 2008). Kinetochores are able to convert the energy from microtubule depolymerization into chromosome movement. The mitotic checkpoint, a process which prevents premature chromatid separation, acts through the kinetochore (for a review, see Musacchio and Salmon, 2007). The checkpoint can detect even a single unattached kinetochore and delay chromatid separation until all are attached. In response to incorrectly attached kinetochores, the checkpoint also induces corrective detachment. As expected for a molecular machine with so many functions, the kinetochore is a focal point for regulation, which occurs through phosphorylation, sumoylation, and methylation of its components. Uncovering how the kinetochore works is central to understanding mitosis.

Spindle microtubules are constantly growing and shortening, and biologists have long wondered how kinetochores stay attached to these dynamic filaments. Time-lapse movies show that kinetochores and their associated chromosomes move continually back-and-forth as the microtubules polymerize and depolymerize under their grip. Several models explaining this dynamic attachment proposed in the 1980s (Hill, 1985; Koshland et al., 1988) are becoming directly testable. Through a combination of genetics and biochemistry, we now know that the kinetochore is a collection of at least 60 proteins arranged into subcomplexes (Cheeseman and Desai, 2008). An increasing number of these subcomplexes can be produced in recombinant form in large quantities, paving the way for biochemical and biophysical interrogation, for structural studies, and possibly for complete reconstitution of active kinetochores from pure components. While a few EM structures are available (Davis and Wordeman, 2007; Wang et al., 2007), atomic-level structural information has been challenging to acquire. Now, in an important advance,

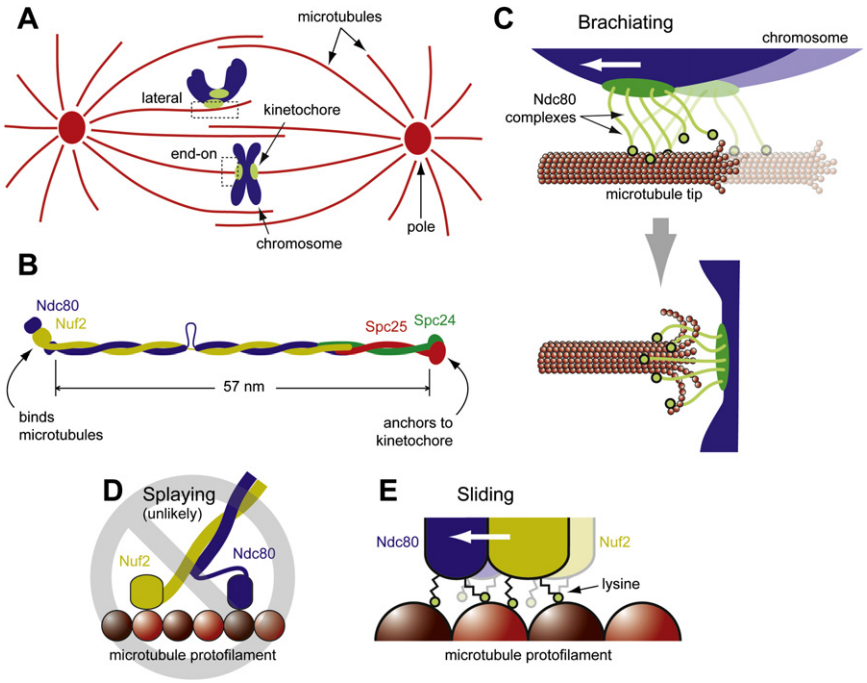

Figure 1. Dynamic Attachment of Chromosomes to Microtubules Depends on the Ndc80 Complex

(A) The mitotic spindle organizes and separates chromosomes during cell division. Microtubule fibers (shown in red) emanate from two spindle poles and attach to specialized sites called kinetochores (green) on each chromosome (blue). Kinetochores initially attach to the sides of microtubules but quickly mature into end-on attachments and thereafter chromosome movement is coupled to the growth and shortening of the attached fibers.

(B) Each Ndc80 complex is a heterotetramer with globular domains at each end of a 57 nm coiled-coil stalk. One end binds microtubules, while the other is anchored to the kinetochore.

(C) Given the multiplicity of Ndc80 complexes and their apparent flexibility, some could bear load while others unbind and rebind in new locations, thereby allowing a kinetochore to brachiate or reorient without detaching from the microtubule.

(D) In principle, the Ndc80 and Nuf2 globular domains could act independently, transiently splaying apart like the heads of a dimeric motor protein. However, the large hydrophobic area of interaction between the Ndc80 and Nuf2 globular domains suggests this is unlikely.

(E) The complex binds microtubules through a large number of flexibly tethered charges, including lysines on the surface of Ndc80/Nuf2 (pictured), other positive charges in the N-terminal extension of Ndc80 (not pictured), and negative charges in the E-hook of tubulin (not pictured). These could allow individual complexes to slide along the microtubule without detaching, similar to DNA-scanning enzymes.

Ciferri et al. (2008) provide the first atomic structure of a core kinetochore component, the Ndc80 complex.

The Ndc80 complex is conserved from yeast to human. It consists of four proteins, Ndc80 (also known as Hec1 in humans and Tid3 in yeast), Nuf2, Spc24, and Spc25 (Figure 1B). The proteins assemble into a heterotetrameric rod about 57 nm long with globular heads at both ends of a coiled-coil (Ciferri et al., 2005; Wei et al., 2005). The complex is a critical component of the core microtubule binding activity of the kinetochore, required both for the initial lateral interaction between the kinetochore and the microtubule (Figures 1A and 1C, top) and for the ultimate end-on interaction (Figures 1A and 1C, bottom). Mutation or depletion of the complex weakens both interactions (Cheeseman and Desai, 2008).

In vitro, when present at saturating concentrations, the Ndc80 complex decorates microtubules like porcupine quills all tilted in the same direction (Cheeseman et al., 2006). Microtubule binding is mediated through the Ndc80/Nuf2 globular region (Ciferri et al., 2008; Wei et al., 2007). Previous structural work showed that the Ndc80 N-terminal region folds into a calponin homology (CH) domain similar to that found in the microtubule binding protein EB1(Wei et al., 2007). In addition to binding microtubules, Ndc80 binds to the Dam1 complex (at least in yeast), which itself binds microtubules. The structure of another piece of the complex was also solved previously. Spc24 and Spc25 form a single globular head with a hydrophobic cleft (Wei et al., 2006). This end of the complex lies toward the chromosome and binds the Mis12/KNL1 complex, which also binds microtubules and enhances the microtubule binding affinity of the Ndc80 complex. The structure of the Nuf2 N-terminal region was unknown.

The structure

Through elegant engineering, Ciferri and coworkers solved the structure of a short ''bonsai'' version of the whole Ndc80 complex (Ciferri et al., 2008). They fused a truncated version of Ndc80 (lacking most of the coiled-coil) to a truncated version of Spc25. Similarly, a truncated version of Nuf2 was fused to a truncated version of Spc24. These two chimeric chains assemble into a stable heterodimer, termed $Ndc80^{bonsai}$. Successful crystallization required additional removal of a presumably disordered N-terminal extension on Ndc80 yielding $Ndc80^{\Delta N\text{-}bonsai}$.

The structure of $Ndc80^{\Delta N\text{-}bonsai}$ reveals important new information about the Nuf2 and Ndc80 globular region. Like Ndc80, the N-terminal region of Nuf2 folds into a CH domain. The Nuf2 and Ndc80 CH domains form a compact assembly maintained by an interface burying a total of 2300 $Å^2$ on each polypeptide. The interface includes the hydrophobic patch previously proposed to represent the microtubule-binding region of another CH domain protein, EB1 (Hayashi and Ikura, 2003). The large area of interaction and its hydrophobic nature suggests that the N-terminal regions of these two proteins interact stably.

Ciferri and coworkers found that $Ndc80^{bonsai}$ binds microtubules cooperatively with a high affinity (K_d of 40 nM). Both the N-terminal extension of 80 amino acids in Ndc80 and the CH domains in Ndc80 and Nuf2 contribute to binding (Ciferri et al., 2008; Wei et al., 2007). The structure revealed that many conserved residues in the Ndc80/Nuf2 CH domains fall on one contiguous face. Mutations in lysines on this face decrease the affinity for microtubules. Changing the charge of the residue while partially preserving the aliphatic chain (as in the mutant K166E in Ndc80) reduces the affinity 5-fold more than a change to alanine. The authors also

show that the negatively charged C-terminal tails of the microtubules (E-hooks) are important for binding. In sum, the data strongly suggest that microtubule binding is mediated by electrostatic interactions.

The Ndc80 complex provides multivalent and flexible connections to the microtubule at several levels. First, the complexes at each kinetochore outnumber the attached microtubules (by approximately 8 to 1 in budding yeast) (Figure 1C; Joglekar et al., 2006). Second, the coiled-coil stalk appears to have some flexibility as suggested by breaks in the predicted regions of coiled-coil, by EM images (Wei et al., 2005), and by the two forms seen even with the foreshortened bonsai version in Ciferri and coworkers' crystals. Third, at the atomic level the binding is mediated through many flexibly tethered charges, including lysines with long aliphatic chains on the microtubule-binding face of Ndc80/Nuf2 CH domains (Figure 1E), negatively charged residues on the flexible E-hook of tubulin, and possibly by the many positively charged residues on the N-terminal extension of Ndc80.

Questions for the Future

Given a high-resolution structure for the Ndc80 complex, a next step is to understand how it fits into the rest of the kinetochore. The structure itself will be invaluable in this regard. For example, mutagenesis will identify residues important for interaction with its known partners. Mapping these residues onto the atomic structure will reveal where the other components are located relative to the Ndc80 complex. Undoubtedly, cryo-EM will provide views of the complex bound to microtubules and other kinetochore components. Docking the Ndc80 structure into these lower-resolution reconstructions will help define the architecture of the kinetochore-microtubule interface.

A difficult problem will be to determine how the Ndc80 complex (together with the other microtubule binding components) enables kinetochores to form attachments that bear load yet move relative to the microtubule lattice. When laterally attached, kinetochores slide along microtubules. A priori, this could be achieved by the Nuf2 and Ndc80 CH domains transiently splaying apart and providing two independent microtubule-binding sites, like the heads of a dimeric motor protein (Figure 1D). However, the extent of the hydrophobic interactions between the two CH domains seems to preclude this possibility. Instead, the tethered charges provided by the Ndc80 complex could mediate a sliding attachment similar to the nonspecific binding of DNA-scanning enzymes (e.g., the Lac repressor) on DNA. These tethered charges could also provide the multiple sites of interaction required for continuous attachment and microtubule-driven movement through a biased diffusion mechanism (Figures 1C and E).

We hope the structure by Ciferri and coworkers is only the beginning. More structures are crucial for understanding how the multiple kinetochore subcomplexes interact with each other, how their interaction is regulated by the spindle checkpoint, and how they allow binding to a constantly remodeling microtubule.

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