The Structure of Purified Kinetochores: From Kinky Rods to Rings

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GMR Deyter

Accurate chromosome segregation is critical for organismal health and viability. Errors in this process are among the leading causes of fetal miscarriages and developmental defects including Down's syndrome. Chromosome missegregation also fuels tumorigenesis. Therefore, it is critical to uncover the molecular mechanisms that guarantee the proper partitioning of the genome during cell division. The laboratory of Dr. Sue Biggins (Basic Sciences Division) has begun to decipher the structure of the intricate protein complex necessary for chromosome segregation.

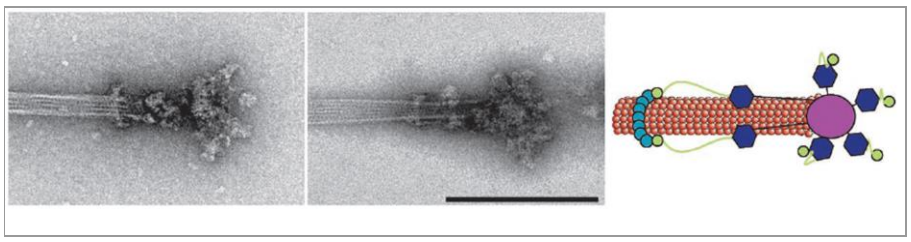
Chromosomes serve as the platform for their own segregation by recruiting a supramolecular protein machine called the kinetochore that interacts with microtubules of the mitotic spindle. Microtubule binding to kinetochores is a dynamic process and is necessary for chromosome movement and segregation into the two daughter cells during mitosis. Thus, there is profound interest in determining how microtubules attach to kinetochores. The simplest kinetochore is found in budding yeast and it attaches to only one microtubule; kinetochores in higher eukaryotes bind multiple microtubules. However, budding yeast kinetochores are thought to represent a fundamental functional unit that is repeated in metazoan kinetochores, thus allowing multiple microtubule binding activities in those organisms. Therefore, insights gleaned from studies of yeast kinetochore-microtubule binding will likely shed light on this evolutionarily conserved mechanism.

Electron microscopic (EM) studies of vertebrate kinetochores have revealed a relatively low-resolution three-layered structure consisting of two 'plates' separated by a space. One plate is proximal to the chromosome with the other plate contacting microtubules. Indeed, kinetochores contain subcomplexes that are responsible for distinct kinetochore activities. Genetic and biochemical experiments have uncovered key inner kinetochore proteins that incorporate into chromatin and interact with DNA. Outer kinetochore complexes interact with microtubules. Three complexes are especially important for interactions with microtubules (termed the KMN network). Although the regulation of the microtubule binding activity of KMN has been studied with recombinant proteins reconstituted *in vitro*, little is known about how this complex functions within intact kinetochores.

Given the plethora of kinetochore proteins, previous attempts to isolate intact kinetochores from cells have been largely unsuccessful and therefore subcomplexes made using recombinant proteins were considered suitable for analysis.
Recently, the Biggins lab devised a method to isolate intact kinetochores from yeast cell extracts. These kinetochores bind microtubules and remain attached to them *in vitro* under forces that kinetochores are subject to *in vivo*. Therefore, these purified kinetochores offer the unique opportunity to visualize whole, functionally active kinetochore assemblies.

The structure of the purified yeast kinetochores was solved by EM in collaboration with the Gonen lab (Janelia Farm Research Campus, Howard Hughes Medical Institute). In the absence of microtubules, the kinetochores appeared as ~126 nanometer (nm) particles consisting of a ~37 nm central hub surrounded by five-to-seven globular domains, each approximately 21 nm. Next, kinetochores were incubated with microtubules to analyze the substructures that mediate microtubule interaction. Small kinetochore particles (likely representing microtubule binding kinetochore subcomplexes) and larger, more intact kinetochores were apparent (see image). The smaller particles bound to microtubules via the globular domains, and a rod-like extension emanated from the globular domain and contacted a ring-like structure that encircled the microtubule. The larger kinetochore structures (containing a central hub with radiating globular domains) also interacted with microtubules through multivalent modes. The globular domains attached to the microtubule, as did the end of the rod-like extensions. A ring-like structure connecting to the rod was often apparent and was always present when kinetochores attached to the tip of a stabilized microtubule, suggesting that the ring has a fundamental role in this important form of kinetochore-microtubule attachment. The Dam1 complex has been shown to assemble rings around microtubules *in vitro* and kinetochores isolated from *dam1* mutants lacked rings, thus confirming the protein components of the ring. Also, previous structural work on the recombinant ndc80 complex (part of the KMN network) revealed a rod-like structure that contained a kink, indicating the potential identity of the rod-shaped complex within the kinetochore. Lastly, the structure of the kinetochore seemed elastic with microtubule binding, suggesting that kinetochores are capable of large conformational changes upon microtubule interaction. Although additional research is required to determine the precise location of specific proteins within the kinetochore EM representations, these data provide the first high-resolution images of the kinetochore, the key complex regulating the crucial process of chromosome segregation.

Gonen S, Akiyoshi B, Iadanza MG, Shi D, Duggan N, Biggins S, Gonen T. 2012. The structure of purified kinetochores reveals multiple microtubule-attachment sites. Nat Struct Mol Biol. Epub ahead of print, doi: 10.1038/nsmb.2358.



Adapted from the manuscript.

Two EM images of kinetochores bound to the tip of a stabilized microtubule. The illustration on the right highlights the key structures involved in kinetochore-microtubule binding. Multiple globular domains (blue hexagons) contact the microtubule (red) and have rod-like extensions that contain a 'kink' (green lines and circle) that associate with the MT-encircling distal ring (blue circles). Thus, kinetochores form multivalent attachments to microtubules. The central "hub" (purple circle) likely corresponds to the chromosome-binding kinetochore subcomplexes. Scale bar = 200 nm.