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One Ring to Bind Them All

Nature's Smart, Elegant Solution to Preserving Genetic Integrity

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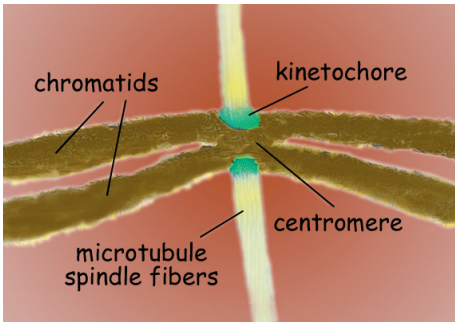
A mystery surrounding tubulin, the protein that plays a crucial role in the passing of genetic material from a parent cell to daughter cells, has been at least partially solved. A team of Berkeley Lab and UC Berkeley researchers has shown that fibers of tubulin—called microtubules—interact with the complex of proteins known as the kinetochore and cause the kinetochore to assemble a ring around these fibers.

The ring, which is attached to the chromatid via the kinetochore, is pushed along the length of the fibers as they break down, carrying the chromosome along without ever letting go. In this manner, the kinetochore ring assures the faithful segregation of chromosomes during cell division.



Kinetochores are attached to either side of a chromosome and ferry it along a microtubule spindle, keeping it segregated from other chromosomes during cell division. Segregation is critical for preventing mistakes that can lead to cancer and birth defects.

"Nature has come up with a smart and elegant solution to the problem of controlling microtubule dynamics during mitosis," says biophysicist Eva Nogales, who holds joint appointments with Berkeley Lab's Life Sciences Division, UC Berkeley's Molecular and Cell Biology Department, and the Howard Hughes Medical Institute, and who led the research.

Mitosis is the process by which a dividing cell duplicates its chromosomes and distributes them equally between its two daughter cells—a process in which mistakes can lead to cancer and birth defects. To ensure the equal distribution of chromosomes, spindles of microtubules attach to a chromosome's centromere through kinetochores. (The centromere is the central region where the two chromatids that make up a chromosome connect.)

It was once thought that kinetochores contain molecular motors that enable them to propel their attached chromosomes along the microtubule spindles. Now it is known that chromosomal separation occurs even if possible motors are removed from the cell—so as long as the microtubules themselves are allowed to remain dynamic.

"When microtubule fibers come to the kinetochore, the kinetochore forms a ring around the microtubule it engages, stabilizing it in the process," says Nogales. "Later in mitosis, when the microtubules are forced by other proteins to break apart, the peeling of the microtubule wall pushes the ring toward each of the two daughter cells. Thus the ring utilizes the breakdown of microtubules as an energy source."

Nogales is an authority on the structure and dynamics of microtubules. For the past three years she has been working with UC Berkeley colleagues Georjana Barnes and David Drubin on a 10 protein kinetochore complex found in yeast, called Dam1. In 2005, the team reported that the Dam1 kinetochore complex formed a ring which promoted the stability of microtubules in spindles and allowed for the continued attachment of microtubules to the kinetochore. Yet at the time, the dynamics behind the ring formation, critical to understanding and possibly exploiting the process, remained unknown.

"Our latest studies suggest that the kinetochore ring is not a preformed structure waiting for the microtubules to pass through its center, but instead is a structure that self-assembles around the microtubules when

continued

Eva Nogales, Hong-Wei Wang, and Vincent Ramey used a combination of cryo-electron microscopy and 3-D image reconstruction to show that when microtubule spindles encounter Dam1 kinetochore complexes, the complexes self-assemble into a ring around the microtubules.
(Photo Roy Kaltschmidt)

they encounter a kinetochore complex," Nogales says. "This explains the mystery of how a microtubule is able to thread its way through a kinetochore ring."

To determine how the assembly of a kinetochore ring is regulated and how the ring attaches to other components of the kinetochore, Nogales and her colleagues used a combination of cryo-electron microscopy and three-dimensional image reconstruction. This yielded structural images of the Dam1 complex before and after the formation of a ring around the microtubules.

The before-and-after images implied that when microtubules encounter Dam1 kinetochore complexes, they induce the complexes to undergo a large conformational change, forming kinks that give them the necessary shape to self-assemble themselves into a ring structure.

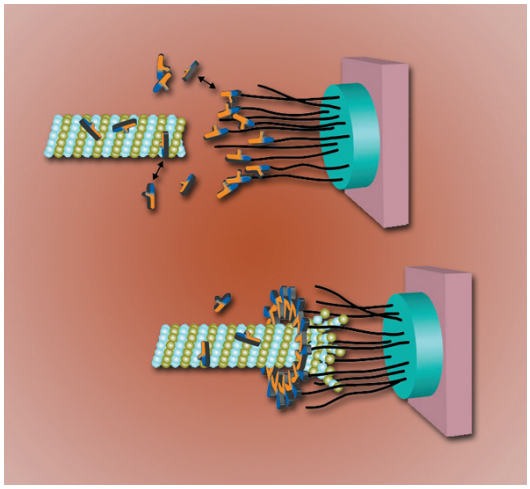
In addition, the researchers found that Dam1 interacts electrostatically with the acidic tails of tubulin but does not have a specific footprint on the microtubule lattice—unlike motor proteins and proteins classically associated with microtubules.

"This tubulin tail is very acidic and it makes a cloud of negative charges around the microtubules, which the Dam1 ring grabs hold of," says Nogales. "This electrostatic means of holding onto the microtubules is not so sticky or tight, but instead allows for lateral sliding of the Dam1 rings along the microtubules."

Nogales and her colleagues also identified a region in Dam1 essential for the regulation of the complex, by spindle-checkpoint kinase enzymes. "These kinases are signaling proteins that, based on tension in the spindles, tell the ring when the time is right for it to let go of the microtubules," Nogales says. "We have found that without this region, the ability of the Dam1 to form a ring is reduced."

Any process that is essential to cell mitosis and could easily be blocked is a potential avenue for the treatment of cancer. While the Dam1 kinetochore has so far only been found in yeast, some of its constituent proteins are common to other eukaryote cells. Furthermore, the biophysics behind the Dam1's interactions with microtubules should have analogies in the cells of other organisms, including humans.

"This is such a beautiful solution to make use of microtubule dynamics that it is bound to be repeated elsewhere," Nogales said. "We have found the first ring; surely there are other rings that await discovery."



Dam1 complex dimers are shown in blue and gold, with some complexes in solution and others associated with the outer region of the kinetochore (black lines). When they encounter a microtubule, the Dam1 complexes self-assemble into a kinetochore ring (bottom). As the microtubules break down, the ring is pushed outward along the fibers toward the daughter cells, bringing along the kinetochore and attached chromosome.

Additional information

"Architecture of the Dam1 kinetochore ring complex and implications for microtubule-driven assembly and force-coupling mechanisms," by Hong-Wei Wang, Vincent H. Ramey, Stefan Westermann, Andres E. Leschziner, Julie P. I. Welburn, Yuko Nakajima, David G. Drubin, Georjana Barnes, and Eva Nogales, appears in the August 1, 2007, issue of *Nature: Structural and Molecular Biology* and is available online to subscribers at http://www.nature.com/nsmb/journal/v14/n8/full/nsmb1274.html.

More on Eva Nogales's research at http://cryoem.berkeley.edu/

More on the research of Georjana Barnes and David Drubin at http://mcb.berkeley.edu/labs/drubin_barnes/