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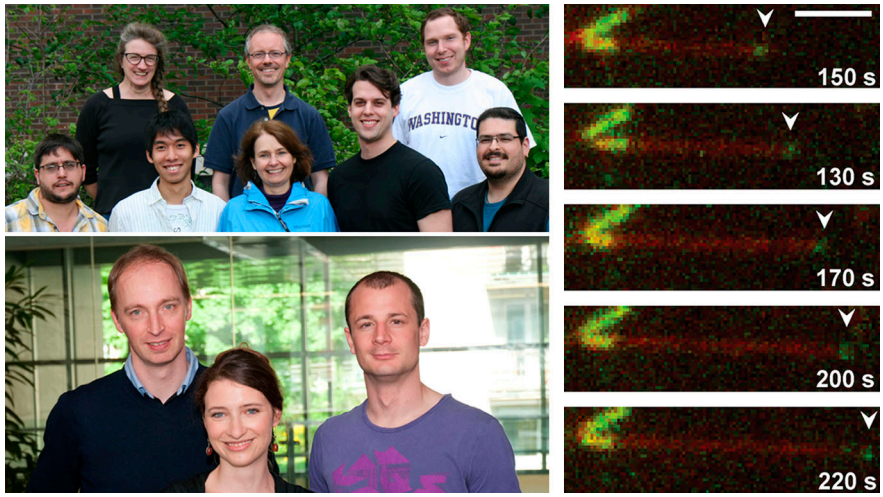
Kinetochores hang on for the ride

Two studies reveal how kinetochores stay attached to dynamic microtubules.

Focal Point



A Seattle-based group led by Trisha Davis and Chip Asbury (top, center), and a Viennese team led by Stefan Westermann (bottom, left) both describe how kinetochores cling to growing and shrinking microtubules during mitosis. The Dam1 complex (green) tracks the dynamic tip of a microtubule (red) and can recruit the kinetochore complex Ndc80 to follow along with it. Ndc80 alone has only a weak affinity for microtubules and fails to track the polymerizing and depolymerizing end in Dam1's absence. The aurora B kinase phosphorylates Dam1 to inhibit its association with Ndc80, which could help eliminate incorrect microtubule–kinetochore attachments.

Chromosomes are shunted around the cell during mitosis to align at the metaphase plate and then segregate to the spindle poles. These movements are driven by dynamic microtubules, which polymerize and disassemble to pull the chromosomes into position. Chromosomes grab microtubules via their kinetochores, but how do they maintain their grip as the microtubules turn over? "It's pretty amazing," says Trisha Davis from the University of Washington in Seattle. "Thousands of subunits come off the microtubule, yet the kinetochore hangs on. And when subunits are added to the microtubule end, the kinetochore stays bound to the tip." Two studies—one from Davis', Chip Asbury's, and Linda Wordeman's groups in Seattle; the other from Stefan Westermann's lab at the Institute of Molecular Pathology in Vienna—describe how two protein complexes combine to keep yeast kinetochores attached to their moving target (1, 2).

Maintaining the link is particularly important for budding yeast, which only connect a single microtubule to each of their kinetochores. "The cell has to avoid losing this microtubule once it's correctly attached, as it would be difficult to re-find it," Westermann says. At least two microtubule-binding complexes attach yeast kinetochores to the spindle—Ndc80 and Dam1. Both are essential for chromosome segregation, indicating that each complex has its own unique function (3).

To find out if either complex attaches kinetochores to dynamic microtubules, the two groups purified Dam1 and Ndc80 proteins and used TIRF microscopy to visualize their interactions with microtubules in vitro (1, 2). The Dam1 complex continuously tracked the growing and shrinking tips of microtubules, while the Ndc80 complex failed to accumulate at these dynamic sites on its own. Ndc80's behavior changed in the presence of Dam1, however. "When we added Dam1, we saw a robust recruitment of Ndc80 to the assembling and disassembling ends," Westermann explains.

> **"Thousands of subunits come off, yet the kinetochore hangs on."**

Dam1 and Ndc80 associate weakly in solution, but microtubules enhanced the complexes' interaction and co-localization at microtubule tips. In turn, Dam1 boosted Ndc80's affinity for microtubules, but Davis wondered whether this had any functional meaning: "Chromosomes are under force [as they're moved about the cell]. Does Dam1 change the force that Ndc80 can withstand?"

Davis and colleagues loaded Ndc80 onto beads—"It's like a simplified chromosome," she says—and measured the force required to pull the beads off microtubules in the presence or absence of the Dam1 complex. Dam1 strengthened the beads' attachment to dynamic microtubules, allowing the beads to resist forces similar to those thought to exist in cells.

Thus, Dam1 helps Ndc80 hold on to growing and shrinking microtubules and to transmit these forces to the rest of the kinetochore to drive chromosome movement. "Dam1 is a specialized tip-tracking protein that is always at the plus end of microtubules, while Ndc80 is a bona fide structural component of the kinetochore," says Westermann. "Together, they establish a link that transmits the force."

This link is regulated by the aurora B kinase, which promotes accurate chromosome segregation by eliminating incorrect microtubule–kinetochore attachments (4). Both groups found that the kinase phosphorylated Dam1 to abolish its interaction with the Ndc80 complex. "If there's a misattachment, the kinase dissociates these parts of the kinetochore, allowing the error to be corrected," Davis proposes.

Davis wants to explore this regulation further, while Westermann is keen to investigate how Dam1 tracks the microtubule ends. The ultimate goal for both groups, however, is to completely reconstitute the kinetochore in vitro. "How do different kinetochore complexes interact," wonders Westermann. "And how do they functionally cooperate to build this complicated molecular machine?"

1. Tien, J.F., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200910142.
2. Lampert, F., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200912021.
3. Tanaka, T.U., and A. Desai. 2008. *Curr. Opin. Cell Biol.* 20:53–63.
4. Cheeseman, I.M., et al. 2002. *Cell.* 111:163–172.