Walk this (and that) way

In one model of spindle extension, a cross-linking motor walks outwards simultaneously on two antiparallel microtubules, thus shoving them apart. The Eg5 kinesin fits that bill, according to Lukas Kapitein, Erwin Peterman, Christoph Schmidt (Vrije Universiteit, Amsterdam, Netherlands), Tarun Kapoor (Rockefeller University, New York, NY), and colleagues. This homotetrameric, bipolar motor walks toward the plus ends of both microtubules that it cross-links.

To visualize the hand-over-hand and foot-over-foot process, the group manipulated surface chemistry to ensure the formation of microtubule–motor–microtubule sandwiches. But 20-nm/s movement in two directions looks like 40-nm/s in one direction. The solution was to drag Eg5-linked microtubules into X-shaped conformations using antibody-coated beads and a laser trap. Motors were then seen to be translocating toward the plus ends of both linked microtubules.

This confirms a “mitotic muscle” model of spindle extension. The model has been around for a long time, and has had candidate mediators in budding yeast, but until now had not been directly visualized. Kapoor says that, for him and his collaborators, “this is just a starting point.” They hope to add opposing, minus-end–directed motors and more complex microtubule arrays to the in vitro assays, with the eventual aim of reconstructing a functioning spindle from known components.

Reference: Kapitein, L.C., et al. 2005. Nature. 435:114–118.

Getting ahead by staying still

Migrating cells send the nucleus backward rather than the microtubule-organizing center (MTOC) forward, say Edgar Gomes, Shantanu Jani, and Gregg Gundersen (Columbia University, New York, NY).

As cells begin to migrate, they spin their internal contents around to orient in the direction of overall cell movement. Movement of a MTOC was thought to lead the way in this process. Consistent with this idea, dynein tugged on MTOCs in other settings, and was concentrated at the leading edge of moving cells. The MTOC moving toward the front of a migrating cell “had been our model forever,” says Gundersen.

The trouble is, he says, “people weren’t looking early enough.” They had seen the final result but not the movement itself. Now, this team gets reorientation going with LPA before initiating migration with serum. That allows them to catch the nucleus moving backward even as the MTOC stays put.

The GTPase Cdc42 and its target MRCK were necessary and sufficient for nuclear movement. These proteins prompt actin polymerization at the front of the cell and thus actin retrograde flow, whose timing and speed match that of nuclear movement. The team favors an actin conveyor-belt model for nuclear movement, but has not ruled out a bulldozer model.

The MTOC stayed fixed in the center of the cell thanks to a group of proteins including dynein, Par6 and PKC/; without them it wandered backward with the nucleus. How the MTOC is fixed in a mystery—some have suggested that the pull of motors on microtubules is proportional to the length of the microtubules.

Reference: Gomes, E.R., et al. 2005. Cell. 121:451–463.

RNA in the machine

Spindle assembly requires a large complex that includes essential RNAs, say Michael Blower, Maxence Nachury, Rebecca Heald, and Karsten Weis (University of California, Berkeley, CA).

The team was searching in frog egg extracts for spindle assembly factors—specifically those downstream of the Ran GTPase. Ran activated by a chromatin-bound GTP exchange factor displaces importin α, an inhibitor of spindle assembly. Known factors downstream of Ran all bind importin β indirectly, via its partner importin α.

Depleting extracts using a mutant importin β gave a nonfunctional extract even though proteins that bind importin α remained. What was missing was Rae1. This importin β-binding protein was previously associated with mRNA export in yeast, and full spindle-promoting activity of Rae1 required a complex of 10 or more proteins and a host of RNAs. Without the RNA, the complex lost several of its proteins and some of its activity. Clusters of RNA were visible in the spindle, and RNase treatment of frog egg extracts inhibited spindle assembly.

“Everyone believed that the spindle was a purely protein-based machine,” says Weis. Identifying the new RNAs will help determine whether they simply hold together a complex or are more active, as in ribosomes and spliceosomes.

Reference: Blower, M.D., et al. 2005. Cell. 121:223–234.