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The spindle plays both ends

In 2003, Khodjakov et al. extended the search and capture model of mitotic spindle assembly.

In order to correctly segregate during mitosis, sister chromatids must attach their kinetochores to stable microtubule (MT) bundles, known as K-fibers, that are connected to opposite spindle poles. In 1986, Marc Kirschner and Tim Mitchison proposed a "search and capture" model in which dynamic MTs emanating from the centrosomes would be selectively stabilized if their plus ends happened to contact and bind to a kinetochore (1). In the following years, researchers observed these search and capture events in mitotic cells, yet it became clear that cells were able to assemble functional spindles in the absence of centrosomes, suggesting that K-fibers could be formed and organized in other ways, too. Accordingly, in 2003, a team of scientists led by Alexey Khodjakov and Tarun Kapoor demonstrated that K-fibers could assemble at kinetochores before their minus ends were captured and incorporated into mitotic spindles (2).

It can be hard to distinguish K-fibers from all the other MTs in mitotic cells. One solution is to study monopolar spindles, in which the chromosomes encircle a pair of centrosomes that have failed to separate. Kinetochores facing the outside of this circle are shielded from the mass of spindle MTs, allowing their association with K-fibers to be observed more easily. Naturally occurring monopolar spindles are rare and transient, but, as a postdoc with Tim Mitchison, Tarun Kapoor had helped identify and characterize a chemical probe, monastrol, that, by inhibiting the kinesin Eg5, blocked centrosome separation and induced monopolar spindle formation (3, 4). "For the first time, we had a small molecule that inhibited spindle organization without directly affecting MT polymerization," Kapoor explains.

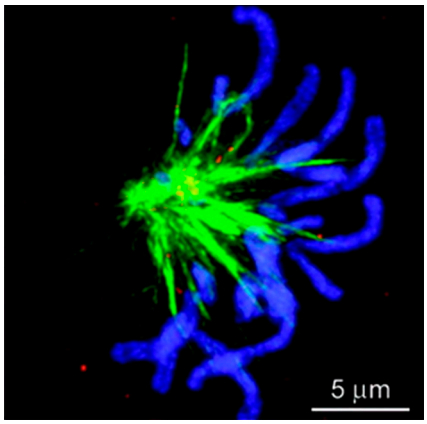
Starting his own lab at The Rockefeller University in New York, Kapoor wanted to use the newly developed technique of spinning disk confocal microscopy to characterize monastrol's effects in more detail. Together with one of his first graduate students, Lily Copenagle, Kapoor saw that, in monastrol-treated cells, MT bundles appeared to extend from outward-facing kinetochores toward the cell periphery before they looped back and incorporated into the monopolar spindle, as if their distal, minus ends were being captured and pulled toward the unseparated centrosomes. For Kapoor, this explained why monastrol induces a high number of syntelic attachments, in which sister kinetochores connect to the same spindle pole.

Meanwhile, at the Wadsworth Center in Albany, New York, Alexey Khodjakov had made similar observations in live, monastrol-treated cells, and, by electron microscopy, had confirmed that these looping MT bundles were indeed K-fibers associated with kinetochores facing away from the monopolar spindle. "There was no way for centrosomal MTs to be captured by these kinetochores," says Khodjakov. "Instead, we saw bundles grow outwards, make a loop, and come back to the centrosomes."

Khodjakov and Kapoor met at a conference and shared their results with each other. "We realized we were seeing the same phenomenon," Kapoor recalls. The two groups combined their efforts and roped in Michael Gordon and Duane Compton, from Dartmouth Medical School, to investigate how the minus ends of these distal K-fibers were captured and pulled toward the centrosomes. An obvious candidate was the motor protein dynein and its binding partner NuMA, which had been shown to focus MT minus ends at spindle poles (5, 6). When Gordon and Compton injected cells with anti-NuMA antibodies, distal K-fibers projected straight out toward the cell periphery and failed to loop back and incorporate into monopolar spindles.

> "We realized we were seeing the same phenomenon."

This NuMA-dependent process of MT capture and incorporation also occurred when monopolar spindles bipolarized after the removal of monastrol. And, by combing through a library of time-lapse recordings, Khodjakov et al. identified two instances of preformed K-fibers incorporating into completely unperturbed spindles. The following year, Khodjakov and colleagues demonstrated that the process significantly contributed to spindle assembly in *Drosophila* cells (7).



In a monastrol-treated cell, some microtubules (green) extend from kinetochores on the distal sides of chromosomes (blue) and, with the help of NuMA (red), loop back and incorporate into the monopolar spindle.

The researchers had therefore extended Kirschner and Mitchison's search and capture model. Kinetochores capture the plus ends of MTs nucleated by centrosomes, while centrosomes capture the minus ends of MTs assembled at kinetochores. The former process is less efficient because kinetochores are small. The latter process, however, is more error prone, because preformed K-fibers can easily be captured by the wrong spindle pole. "Cells want to build a spindle quickly, but with a minimal amount of errors," Khodjakov says. "The relative contributions of these two assembly mechanisms remain unknown."

Khodjakov and Kapoor, who have continued to collaborate over the last 12 years, are interested in resolving this question and in determining why K-fibers are so much stabler than other spindle MTs.

"This landmark paper beautifully illustrated an elaboration of 'search and capture' that no one had seen before," says *JCB* academic editor Rebecca Heald. "It's also a terrific example of the strong collaborative spirit that characterizes our field."

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