Motors or dynamics: What really moves chromosomes?

J. Richard McIntosh

Since the discovery of mitotic spindle microtubules, there have been two competing ideas about the origins of forces for chromosome segregation during cell division: the dynamics of microtubules, and the action of motor enzymes. When I entered this research field in the late 1960s, the sliding filament theory of muscle contraction was already well established, and contemporary structural and biochemical work on cilia made a motor-based mechanism for mitosis seem likely. Moreover, my colleagues and I discovered a remarkably simple model for mitosis based on the postulate of a single, microtubule-sliding motor and some — then plausible — assumptions about spindle microtubule polarity. So in spite of the beautiful work by Shinya Inoue and his students showing the importance of microtubule depolymerization for permitting chromosome motions, I was committed to the idea that motors were the drivers. Microtubule dynamics might be a mitotic regulator, but they seemed unlikely to provide the motive force.

Evidence for this position mounted during the 1970s and 1980s. Electron microscopy demonstrated rearrangements of spindle microtubules consistent with the idea that spindle elongation was driven by microtubule sliding. Experiments on spindle 'models', made by lysing cells in buffers that maintained spindle structure, showed that adenosine triphosphate (ATP) was required for interpolar microtubule sliding, as happens during spindle elongation. The discovery of the kinesin motor and the determination of its sequence led to the realization that several mitotic mutants in fungi carried genes encoding defective kinesin-like proteins. The localization of dynein to kinetochores, where this pole-directed, microtubule-dependent motor could help move chromosomes to the spindle poles, enhanced the case. Although an assay our lab developed for microtubule polarity demonstrated that the simple model for mitosis was wrong, some spindle motions really did seem to depend on motors. Why not all of them?

In 1988, however, Marc Kirschner's group published a provocative paper in *Nature* entitled 'Polewards chromosome movement driven by microtubule depolymerization *in vitro*', which showed how controversial the issue remained. Discussions in our lab led me to believe that it was imperative to develop an assay to look directly at the ability of microtubule depolymerization to exert forces on chromosomes. Martine Coue and Vivian Lombillo, a postdoctoral researcher and graduate student in our lab, took this project on. Using chromosomes isolated from chinese hamster ovary cells and tubulin purified from bovine brain, they constructed chambers for light microscopy in which the pole-facing ends of many labile microtubules were tethered firmly to small regions on the coverslip, and chromosomes were attached at or near the free microtubule plus ends. With this set-up, and in collaboration with Curt Pfarr and Corey Nislow, Martine and Vivian explored the conditions necessary to make chromosomes move. I had expected that motion would require Mg–ATP, and that we might even be able to identify the motor that did the job. I was amazed to learn that ATP was not required, and that function-blocking antibodies to dynein had no effect on the rates of movement seen. All that was needed was tubulin depolymerization from the chromosome-associated microtubule ends. The only plausible source of motive force in this assay was tubulin depolymerization itself, so we had to conclude that microtubule dynamics could indeed generate chromosome motion.

These results changed my approach to mitotic mechanisms. They did not negate the evidence that motors were important for aspects of mitosis, but they suggested that chromosome motion to the spindle poles could be motor-independent. So how did the mechanism work? These initial experiments defined several lines of work that have occupied our group ever since. Are microtubule-dependent motors dispensable for chromosome-to-pole motion *in vivo*? What molecules serve as tethers to couple the 'payload' to tubulin depolymerization? How much force can a depolymerizing microtubule generate? How is depolymerization regulated, so chromosomes move at their normal, stately pace?

Katya Grishchuk in the lab made a strain of fission yeast in which all the genes for pole-directed motor enzymes were deleted, yet the chromosomes continued to move polewards with a final speed no different from in wild-type cells, a result that has been extended to budding yeast by Tomo Tanaka's group (University of Dundee, Scotland). Many labs have now identified kinetochore proteins that are essential for the formation of successful linkages between chromosomes and spindle microtubules, and our group has explored the biophysical properties of several of these, testing their ability to couple chromosomes to shortening microtubules. Some motor enzymes will do the job, even if they have to be pushed backwards by the depolymerization reaction. Some non-motor molecules will also work: protein complexes that will assemble into rings, proteins that bind to microtubule walls, and even some that bind the flaring strands of tubulin at a microtubule's depolymerizing end. Moreover, depolymerization can generate plenty of force. We can now envision mechanical parallels between mitosis in eukaryotes and chromosome segregation in bacteria, where fibre-dependent motors do not exist but fibre dynamics seem important. The Kirschner paper and our initial system for depolymerization-dependent motility were turning points that led our lab in exciting directions that I would never have anticipated during the days of my fascination with motors.

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