Mitosis futures: the past is prologue

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ABSTRACT **The mechanisms by which cells organize and segregate their chromosomes have been under close scrutiny for years, and significant progress has been made in understanding how mitosis works. Modern cell biology has identified most of the molecules that underlie mitotic spindle function, but the ways in which they are organized and controlled to make an effective and accurate cellular machine are exciting subjects for future study.**

A BRIEF HISTORY OF RECENT RESEARCH ON MITOSIS

Although the elegant and complex motions of mitotic chromosomes were initially mysterious, work from a legion of researchers has now demonstrated that the segregation of duplicate genomes is accomplished by a spindle-shaped array of microtubules (MTs) that works in collaboration with a coterie of associated proteins and regulatory factors. In a sense, the mechanism of mitosis is solved: the spindle does it. However, students of this subject are now working to understand just how the spindle can do such a complex job. Major progress has been made in the 50-plus years since the discovery of spindle MTs and the early descriptions of spindle behavior in living cells by phase and polarization microscopies. Recently improved microscope technologies, such as fluorescence optics and cameras with improved sensitivity and signal-to-noise ratios, plus reliable preparative methods for both immunofluorescence and electron microscopy, have provided high-quality descriptions of many mitotic structures and events. For example, we now know not only about chromosome motion but also about the motions of spindle MTs (Yang *et al.*, 2008), which turn out to be critically important for understanding how chromosome segregation works. These data have revealed the complexity of mitotic phenomenology, and as a result they have stimulated realistic thought about the molecular mechanisms of mitosis. Gone are the simple models based on electrostatics, magnetic fields, or even on simply MT dynamics (Inoue *et al.*, 1975) or a single motor enzyme (McIntosh *et al.*, 1969). Progress in understanding mitosis is now based on the more solid foundation of what the spindle is actually doing.

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As with other aspects of cell biology, however, descriptions are not sufficient; one needs to know how individual processes work and how they are controlled. Here conventional biochemistry has been of less help than it was in understanding muscle contraction because spindles are small, complex, and labile. Major progress in understanding the molecules that matter for mitosis has come from a loose federation of three approaches: immunolocalization of components identified elsewhere, for example, some MT-associated proteins and the dynein motor enzyme; genetic/molecular analyses of mitosis in organisms suitable to the task (mostly fungi, flies, and worms; more recently, mammals); and finally from biophysical cell biology. All of this work has given us a cast of characters that is getting pretty complete. For example, "kinetochores," the specializations that attach chromosomes to spindle MTs, are now known to contain protein complexes that include motor enzymes, multiple MT-binding proteins, several regulatory kinases and phosphatases, and a signaling device that controls mitotic progression (reviewed in Welburn and Cheeseman, 2008). Certainly there is more to learn about spindle composition, but the focus of current research is to figure out how the known spindle parts work together to make a micromachine (something that can move whole chromosomes over distances as large as half a cell's

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Abbreviation used: MTs, microtubules.

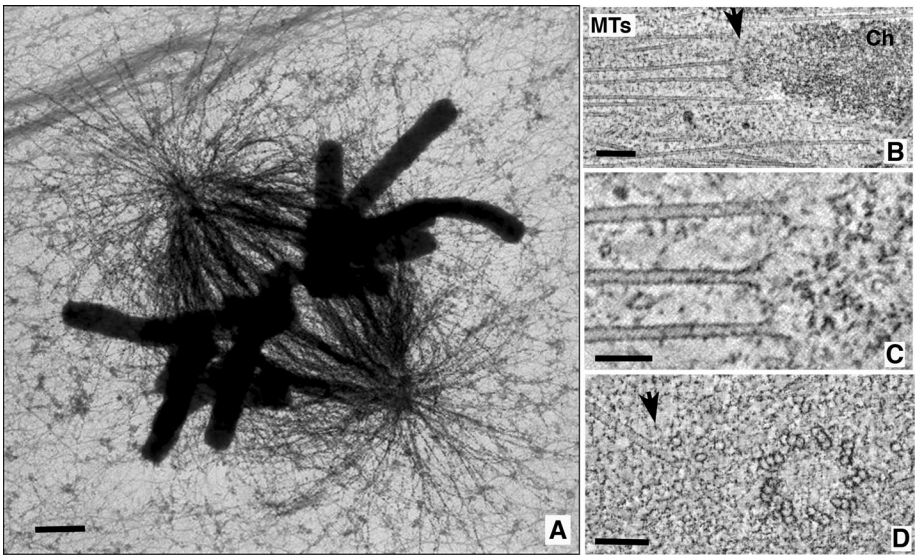

FIGURE 1: Electron micrographs of spindles from mammalian cells, strain PtK1. (A) Cells were cultured on Formvar-carbon–coated gold grids, permeabilized with 0.1% Triton X-100, fixed in formaldehyde/glutaraldehyde, and then stained with a primary antibody against tubulin and a secondary antibody conjugated to colloidal gold. The metaphase cells were imaged in a JEOL1000 electron microscope operating at 1000 keV. Bar, 1 μm. (B–D) Cells were cultured on chips of plastic, cryoimmobilized in a Bal-Tec AG high-pressure freezer, and then fixed by freeze-substitution at –90°C in acetone containing glutaraldehyde and tannic acid, followed by OsO_4 and uranyl acetate; embedding was in Epon-Araldite. Thick sections (~250 nm) were imaged as tilt series in an FEI F30 microscope and tomograms generated and studied using the IMOD software (Kremer *et al.*, 1996). (B) A 40-nm-thick slice from a prometaphase cell in which a chromosome (Ch), microtubules (MTs), and a kinetochore (arrow) are evident. Note its "trilaminar" structure with MTs ending in the outer plate. Bar, 200 nm. (C) A 4-nm slice of the same kinetochore, revealing the flared structure of MT ends at the kinetochore and fibrils that appear to connect these ends with chromatin. The outer plate is not visible here, probably because it is made largely from the flared MT ends, clearly visible in this thin slice. Bar, 100 nm. (D) The polar end of a metaphase MT. This end too is open and flared, suggesting that any γ-tubulin cap has been lost (arrow). Bar, 100 nm.

diameter) out of an army of nanomachines (macromolecules that work as energy transducers, links, and governors).

My lab has contributed to this work in part through a study of spindle structure (Figure 1). I was inspired by Hugh Huxley's work on muscle to believe that fine-structural maps of spindle components, taken as a function of time in mitosis, would provide a foundation for understanding spindle action. Over the years, this work has taken several forms, ranging from quantification of MT numbers as a function of spindle position and mitotic stage, through the three-dimensional tracking of spindle MTs by serial sections or stereomicroscopy of thick samples, and on to the use of electron microscope tomography to get accurate maps of MT trajectories. Many kinds of small spindles, for example, algae (McIntosh *et al.*, 1979), slime molds (McIntosh *et al.*, 1985), and fungi (Winey *et al.*, 1995; O'Toole *et* al., 1999; Grishchuk *et al.*, 2007), have been elucidated in these ways, both in our lab and in others, but the tasks of understanding larger spindles, like those in the cells of fruit flies, vertebrates, and higher plants, still lie ahead. The images now available provide a framework for understanding mitotic processes, but they have not been as informative mechanistically as Huxley's muscle reconstructions. This is partly because spindles are big and disorganized compared with a sarcomere, but also because spindles form and disassemble as they function. Static images miss some of the key dynamism of the events under study, such as the polymerization processes that accompany MT sliding as a spindle elongates.

Our lab has therefore worked to understand spindle dynamics, using fluorescent proteins microinjected into living cells (Salmon *et al.*, 1984; Saxton *et al.*, 1984) and antibodies to known or suspected spindle enzymes (Vaisberg *et al.*, 1993). This work extended earlier ideas about the rapidity of spindle MT dynamics; it also implicated dynein in aspects of spindle formation and function. However, the difficulty of function-blocking experiments by antibody injection led me to pursue a genetic organism with an orthodox spindle as a way to improve the quality of the experimental work. With help from Paul Nurse's lab, we began a study of fission yeast and were able to explore the roles of many mitotic motors in this wonderful organism. It was illuminating, if discouraging, to find that many motors localized to spindles were not essential for spindle function (Troxell *et al.*, 2001; West *et al.*, 2002), but this work culminated in the discovery by Katya Grishchuk that the final motions of chromosomes to spindle poles (movements toward MT minus ends) did not depend on any of the cell's minus end–directed motors (Grishchuk and McIntosh, 2006). This result complemented earlier work from our lab showing that tubulin depolymerization could move mammalian chromosomes in vitro without the benefit of soluble nucleotide triphosphate (Coue *et al.*, 1991; Lombillo *et al.*, 1995).

These results motivated our lab's recent collaborations with Fazly Ataullakhanov from the Physics Department at Moscow State University (Moscow, Russia) on the mechanisms by which MT depolymerization might generate mitotic forces (Grishchuk *et al.*, 2005). For someone who was initially convinced that mitotic motors were important, this has been a bemusing change of scene. We have now shown that tubulin depolymerization can generate sufficient force to move chromosomes (Grishchuk *et al.*, 2008), but the issues of control still lie ahead. Evidence from many labs has shown the importance for mitosis of kinesin-13s and 8s, enzymes that can promote tubulin depolymerization. It remains to be discovered how motors and MT dynamics work together to achieve accurate chromosome segregation. This is one of the most interesting challenges in the field of mitotic research.

WHERE THINGS ARE HEADED

It seems likely that most of the protein components of mitotic spindles are now known, but our understanding of how these molecules work together is still imperfect. Many labs are interested in the roles of mitotic kinases, like Aurora B, for fixing spindle mistakes and helping to get chromosomes properly attached to the spindle. These are key issues that merit close study, both for understanding the basics of mitosis and for dealing with the ways in which chromosome segregation fails, producing aneuploidy. Such work may also lead to novel cancer therapeutics, which could be of significant medical value. My thinking, however, is still focused on the mitotic machinery itself: how mitotic forces are generated and controlled. We know that tubulin depolymerization can generate

force, but how this engine is coupled to chromosomes is imperfectly understood. The connection can bear force, can support both addition and loss of tubulin, and can inform the cell when chromosomes are properly attached, the signal that allows anaphase to start (McIntosh, 1991). Each aspect of this remarkable process can and must be reconstructed in vitro from purified components before we will really understand how cells do these complex jobs. Thus, one genre of work that should be valuable for many years to come will involve the development and characterization of experimental systems in which accurate measurements can be made on the in vitro function of well-characterized protein complexes. Yes, these results will have to be related back to a living cell, but the complexity of mitosis suggests that a "divide and conquer" approach will provide important insights for understanding mitotic machinery in cells.

WHY STUDENTS OF MITOSIS ARE LUCKY

I have been working on mitosis for ~45 years, and even though many mitotic mysteries have been solved, I still find the process completely fascinating. I share with other students of this amazing cellular event a sense of awe at its effectiveness and precision, as well as a deep admiration for the beauty of its mechanisms, to the extent that we understand them. I and my colleagues in this field have found that each technological advance brings a new slant on mitotic events, allowing a rejuvenation that is refreshing in today's competitive scientific world. I hope others will come to join us as we work toward a better understanding of one of the cell's most spectacular processes.

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