Romancing mitosis and the mitotic apparatus

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ABSTRACT

One of the earliest lessons students learn in biology is the process of mitosis and how cells divide to produce daughter cells. Although first described more than a century ago by early investigators such as E. B. Wilson, many aspects of mitosis and cell division remain the subject of considerable research today. My personal investigations and research contributions to the study of mitosis were made possible by recent developments in the field when I began my career, including access to novel mammalian cell culture models and electron and fluorescence microscopy. Building upon those innovations, my laboratory and other contemporary investigators first charted the ultrastructure and molecular organization of mitosis and chromosome movement and the assembly and function of the cytoskeleton. This field of research remains a significant challenge for future investigators in cell biology and medicine.

MITOSIS, EARLY ENCOUNTERS

I first encountered a microscope in the early 1950s as a freshman biology major. The instrument was an old brass student microscope that we were instructed to use to identify and examine the stages of mitosis in cells of the onion root tip. I was fascinated. It was then that I became more curious about chromosomes, how they attach to the spindle, and how they move through each of the mitotic stages. At that time, the study of mitosis was largely descriptive and limited to light microscopy. Photomicroscopy was still in its infancy, and most published illustrations were hand-drawn images made using a “camera lucida,” an apparatus with a pair of small mirrors attached to the microscope oculars that projected an image onto the desktop at the base of the microscope. Thus the image could be traced in pencil or ink, enabling the observer to accurately measure and record chromosomes and associated structures in dividing cells. Later in my master’s-degree research, I was able to acquire a more advanced research microscope equipped with bright-field and phase optics with 50× and 100× oil-immersion lenses sufficient to study mitotic chromosomes in the neurons of larval mosquito brains. Although the optics were improved, we had no cameras, and my illustrations and measurements were still recorded using a camera lucida. Even so, I was able to make accurate measurements and drawings of metaphase chromosomes from various species for my study of mosquito taxonomy and speciation. I confirmed, as previously documented, that homologous chromosomes of mosquitoes and other dipteran insects remained paired during mitosis. My fascination and curiosity about mitosis and chromosomes grew from those early encounters, and I wanted to pursue this topic further for my doctoral degree.

I decided to pursue my PhD degree at Iowa State University in the early 1960s because the college was one of the first to establish a new graduate curriculum entitled “Cell Biology” that included training in electron microscopy. During this period, research in the cell sciences was advancing at an accelerated pace and beginning to move into more molecular and analytical realms. Of particular interest was the emergence of new analytical instruments, including electron optics, and reports of novel research on mitosis in a variety of systems, including marine organisms, insects, plants, and animals. I was especially fascinated by the innovative use of time-lapse movies to capture mitosis in live cells. Also, electron microscopy...
was becoming a more widely used research tool for studies of cell division. Remarkable experiments were just underway involving the use of micromanipulation techniques with fine needles to probe into cells and actually hook onto chromosomes to measure the minute spindle forces that act upon them in insect cells (Nicklas and Staehly, 1967). Clearly, discoveries in cell research were accelerating. An exciting new era of experimental cellular and molecular biology had dawned, and with it began a new professional organization known as the American Society for Cell Biology. It was clear to me that it was an auspicious time to enter the field of cell science.

After completing graduate school and receiving my PhD degree at Iowa State University in the mid-1960s, I was anxious to pursue postdoctoral research on the molecular basis of mitosis and chromosome movements in mammalian cells. Specifically, I wanted to gain expertise in the biomedical sciences, with emphasis on mitosis and chromosomes in both normal and neoplastic cells. For this, I needed access to cancer cells and tissue culture model systems. I was fortunate in this regard to be accepted as a postdoctoral student in the laboratory of T. C. Hsu, a distinguished expert in chromosome biology at the University of Texas M. D. Anderson Hospital and Tumor Institute in Houston (currently known as the University of Texas M. D. Anderson Cancer Center). There, I soon met and began collaborating with his team, a highly motivated group of colleagues with wide-ranging expertise. From them, I learned the fundamental methods of mammalian cell culture. I learned how to synchronize the growth of cultured cells by arresting and collecting cell populations at specific time points in the cell cycle, including mitosis (M phase), G1, S, and G2 phases. In addition to his dynamic team, Hsu’s lab housed an unparalleled collection of unique mammalian cell lines stored in his –80°C freezer, known as “Professor Hsu’s frozen zoo.” For the first time, I could carry out experiments on the mitotic apparatus in animal models uniquely suited for this purpose, with low numbers of chromosomes that were unusually large. I selected Chinese hamster cells, because they could be synchronized and harvested at precise stages of the cell cycle, especially mitosis. In addition, I wanted to investigate rat kangaroo cells with karyotypes containing only 11 chromosomes. I also had access to an even more fascinating cell line derived from the Indian barking deer (Muntiacus muntjac) with a diploid chromosome number of 2N = 6 in the male line and 2N = 7 in the female.

The resources of Hsu’s lab opened seemingly endless opportunities for me as the only team member trained in electron microscopy. I enjoyed early success in characterizing the structure and organization of specialized regions of mammalian chromosomes such as primary constrictions, centromere and kinetochore structures, and secondary constrictions, including nucleolar organizing regions and telomeres. Our most significant early accomplishment was to provide the first detailed EM images of the kinetochore on mammalian chromosomes (Brinkley and Stubblefield, 1966). Following our first publication describing the trilayered plate-like structure and fibrous corona, similar observations have been widely reported on mitotic chromosomes of many eukaryotic organisms. Thus the design of the kinetochore (Figure 1) is widely conserved in eukaryotic cells. There is still much to be learned, however, about this specialized chromosomal component and its function in partitioning chromosomes and maintaining genomic and genetic stability. Many studies are currently underway worldwide.

**LIGHTING UP THE CYTOSKELETON**

My laboratory’s second major accomplishment was to develop the first antibody against tubulin and use it as a fluorescent probe to “illuminate” the microtubule cytoskeleton in mammalian cells. With this discovery, along with similar reports from other labs, began a dynamic era of research on the cytoskeleton. I gladly share the credit for developing this tubulin antibody with my former colleague at the University of Alabama, G. M. Fuller. Working in collaboration with me, Fuller and his students produced the first monospecific antibodies against bovine brain tubulin (Brinkley et al., 1975; Fuller et al., 1975). This significant achievement provided a vital new tool for the detection and analysis of microtubules in mammalian cells. When we began this collaboration, I questioned whether a useful antibody to 6s tubulin could be produced by the techniques available at that time. My lab had tried before and failed. Undaunted, Fuller and his students proceeded to inject rabbits with 6s tubulin purified from bovine brain tissue. When he tested the affinity-purified antisera by staining a monolayer of mouse 3T3 cells, we were delighted that the new antibody stained mitotic spindles. However, to our surprise and initial concern, we also observed numerous brightly fluorescent fibers coursing through the cytoplasm of every interphase cell. Initially, we feared that our new probe might be cross-reacting with another cytoskeletal component, perhaps intermediate filaments. Yet further tests confirmed that our tubulin antibody was highly specific.
for tubulin and microtubules. Our new probe had illuminated an elaborate array of cytoplasmic microtubules heretofore undetected. We named this interphase network the “cytoplasmic microtubule complex” or CMTC. Following a series of champagne toasts to celebrate our success and discovery, I placed a call to the discoverer of microtubules, Keith Porter. He immediately invited us to Boulder, Colorado, to share our findings. Just when we published our initial report in Science in 1975 (Fuller et al., 1975), several other laboratories in the United States and Europe began to report similar results. The era of the cytoskeleton had begun and continues unabated today.

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