

Mitosis – The story : Conly Rieder of the Wadsworth Center, NY, USA interviewed at the University of Exeter, UK, by Herbert Macgregor and James Wakefield, October 2010

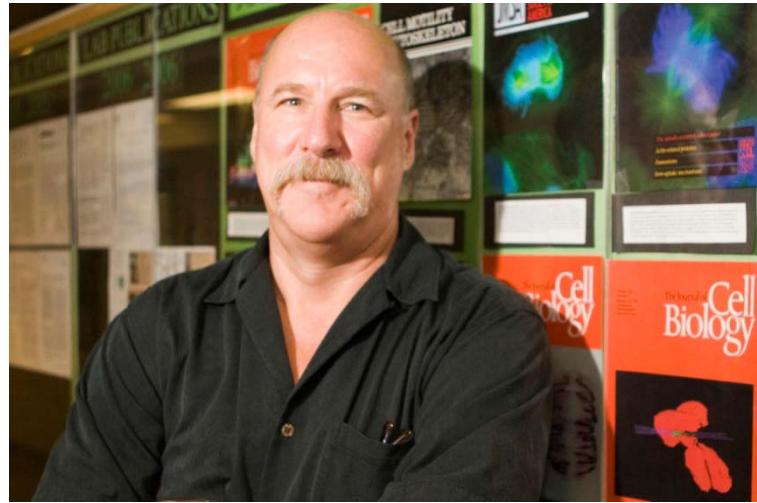
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Conly Rieder has an established reputation not only as a World leader in research into the mechanisms of mitosis and cell division but also as an extraordinary modern microscopist. The sheer artistry and beauty of his portrayal of chromosomes and mitotic spindles as well as the amazing resolution and clarity of his electron micrographs sets a tough standard for any who aspire to follow in his footsteps. Coupled to these exceptional technical skills, Conly is a keenly logical and critical experimentalist and young people entering the field of cell science can learn a lot from the manner in which he has approached the unknowns of the mitotic cell cycle.

Conly spent the latter half of 2010 working in Bill Earnshaw's laboratory in Edinburgh, Scotland. His visit coincided with our decision to prepare this Special Issue of *Chromosome Research* on Mitosis, so we took advantage of the opportunity to spend a couple of hours talking to him with a digital recorder on the table. What follows is a slightly edited version of that most enjoyable conversation.



(fig 1)

Conly Rieder 2010

Were you always inclined to be a biologist?

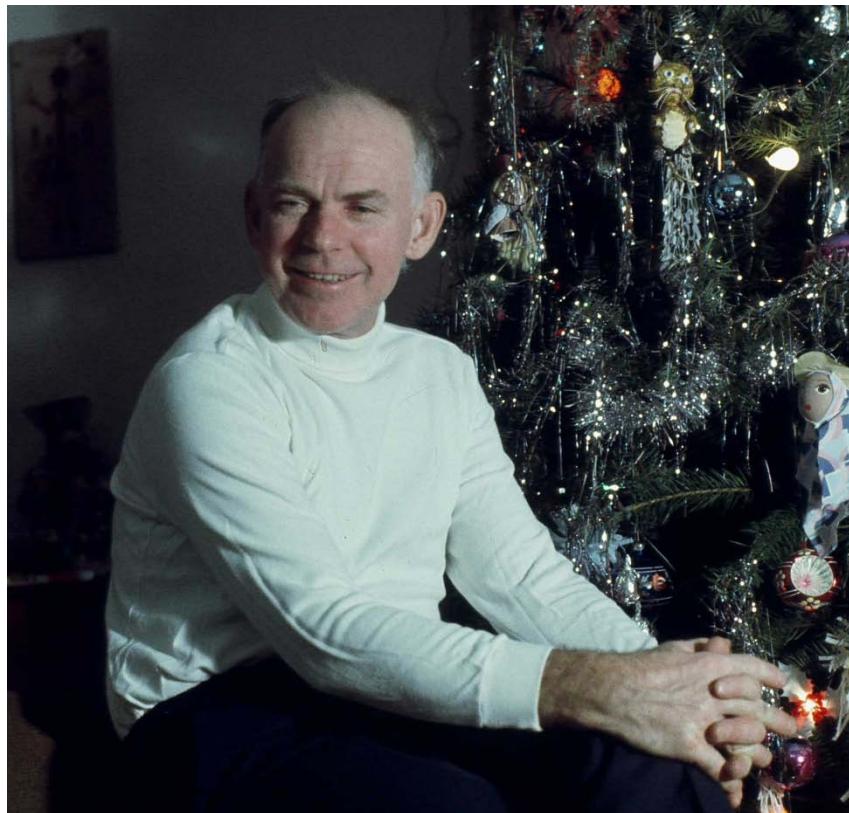
Probably, but the passion took quite awhile to bloom. I was born in southern California and spent the majority of my first 22 years in and around the beach cities—mostly outdoors. When I was in high school I developed a real liking for biology which was my strongest subject. At that time I was mainly interested in forestry—because I loved hiking and fishing in the mountains and the environment.

When I graduated from high school in 1968 I had a choice - either be drafted into the army or go to college: no in between option. I enrolled in the University of California, Irvine, which was a brand new campus 7 miles from home. At the time it I had no clear idea of what to study so I opted for my strong suit. My dad was a retired Marine fighter pilot who was vehemently against the war in Vietnam, and he offered to cover my car insurance and tuition which was \$57 a quarter if I paid for my room and board expenses. I worked full time

while attending Irvine, first in a fast food restaurant and then in a medical library. Working and going to school full time was no fun but it taught me time discipline that came in very handy later. Three years into UCI's Biological Sciences programme I still had no idea what I wanted to do so I decided to apply to medical schools like most of my friends—but I didn't get into a single med school. Lucky for me I backed up my med school attempts with applications to graduate school which I understood was free if you were accepted into a Ph.D. programme. I applied to several programmes, got into all of them, and chose the University of Oregon at Eugene. At the time I did not know what I wanted to work on, or even whether I would complete the program. However, I felt that if I was going to get paid to go to school in a region which was known for its world-class skiing and fishing, I'd be a fool not to at least give it a shot. Although I was still keen on forestry and ecology, I was also very interested in electron microscopy (EM) and cell structure, visual topics on which I did some independent work in my last year at Irvine.

How did you develop an interest in microscopy and then later in mitosis?

In 1972 Andrew Bajer, who was born and educated in Poland, was conducting same-cell correlative light and EM studies on mitosis in *Haemanthus* endosperm at the University of Oregon. This laborious technique entailed using light microscopy (LM) to film a dividing cell and then rapidly fixing it at a desired stage for a subsequent serial section EM analysis. I wrote to Andrew the summer I graduated from UCI and said I was interested in structural biology and EM and that I had been accepted into the U of O and would be interested in working with him. I had excellent grades, a decent Graduate Record Exam score, and good letters of recommendation. I added that I could not come unless I received a fellowship. Andrew was friends with the highly regarded *Drosophila* geneticist, Ed Novitski, and between them and the Dean, Aaron Novick, they came up with a four year post as a teaching assistant. That fall I appeared at Andrews's lab door and introduced myself. The first words out of his mouth as he stared at my chest (he was about a foot shorter than I was) were "I thought you were a woman"! Nevertheless he let me join his lab which at the time consisted of his wife (Jadwiga Mole-Bajer) and two female colleagues, one from France (Anne-Marie Lambert) and one from Spain (Consuelo de la Torre). My first big mistake as I started in his lab was to mention that I typed 80 words per minute—which immediately made me his editor and typist for the next 5 years.



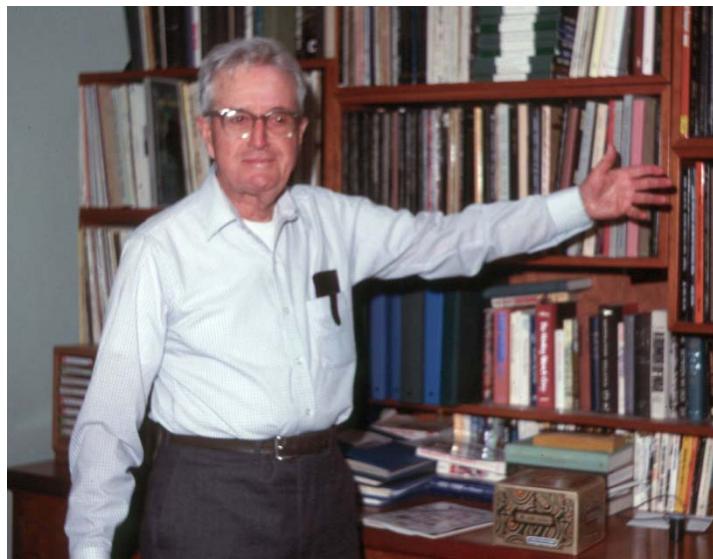
(fig.2)

Andrew Bajer at home Christmas 1995

Several months later I met with my advisory committee to define deficiencies in my background. They advised me to take a range of graduate courses during my first year, from ecology and histology to physical chemistry. They also assigned me my teaching duties, most of which were concerned with preparing and supervising laboratory practical classes. The first year was mostly taken up with teaching and classes, which included a lab-based course in EM. The next 3 years were a mix of teaching and research.

Andrew had made his international reputation primarily on his award-winning time-lapse cinematographic movies of mitosis – some of which are still available. I found genetics, biochemistry and molecular-biology too detached and abstract, but I thought watching and documenting the various dynamic behaviours exhibited by cells was very cool. And the prospect of actually getting paid to learn LM and EM was very appealing. My goal at that time was simply to publish a paper, finish graduate school, and worry about the future later.

My dad had a favourite saying which was “bloom where you are planted”, and I was now firmly planted in the biology department in Eugene, Oregon. Fortunately, James (Jim) Kezer worked right across the hall from Andrew Bajer.



(fig.3)

Jim Kezer at his home ca. 1980

Jim was a very approachable zoologist with an expertise in amphibian cytogenetics. His passion was for opera, salamanders, newts and anything related to the nucleus, often in partnership with colleagues like Joe Gall and Herb Macgregor. I never met Joe or Herbert while at Oregon, but Kezer talked about them all the time. Jim's infectious enthusiasm was definitely a major influence on my career. He taught me histology and nuclear cytology with a strong emphasis on lampbrush and polytene chromosomes. I was inspired by those classes and became good friends with Jim, and during the next two years I was also his teaching assistant for both classes. During the middle of my second year a fellow by the name of Takeshi Seto joined Jim's lab on sabbatical from Japan. Takeshi was an amphibian cell culture expert and he taught me how to grow lung cells from the local newt (*Taricha granulosa*) which was abundant in the cool lakes and wet forests of Oregon. He thought they would be excellent material for studying mitosis because newts have some of the largest chromosomes in the animal kingdom and their lung cells are gigantic, grow very flat, and are optically clear. Although Andrew was a botanist who worked with plant endosperm, he thought it would be a good idea for me to work on mitosis in newts, which unlike *Haemanthus* are not seasonal. For the next 3 years I spent 70 hrs a week typing, culturing newt cells and filming 16-mm movies of mitosis. During this time I digested Andrew's 1972 book on mitosis while babysitting my microscope and I also read as many other papers and books related to cell division as I could, starting with E.B. Wilson's first edition (1896) classic “The Cell in Development and Heredity”. All this reading prompted me to write a library-based Master's thesis in 1975 on how chromosomes move during mitosis. One motivation for getting my M.S. was that I got paid more money as a teaching assistant.

There were positive and negative aspects of working in Andrews's lab. It forced me to become an independent thinker and resourceful experimentalist. Other individuals were more influential on my graduate experience including Kezer and Robert Hard, the latter of whom joined Andrew's lab as a post-doc during my

third year. Andrew hated teaching. He viewed it as an unwelcome distraction from his research, and he used to start his classes at 8a.m. sharp in Polish—few students lasted past the first week. To him, Ph.D. students were an obligation, not a passion, and he preferred working more with women than men. Throughout my 5 years in his lab he never took me to a scientific meeting because he said my hair was too long—and he insisted that I call him “Dr. Bajer” and not Andrew until I got my Ph.D. Besides asking me to type manuscripts, he left me mostly alone with instructions to approach his wife with any questions. Jadwiga (Visha) was an outstanding scientist in her own right and was the “hands” of the Bajer/Mole-Bajer team. She became a strong ally and graciously provided me with the supplies and tools required to do my work. It was a real learning experience working in Andrew’s lab, which was a staging ground for many very interesting events – some really, really funny and some not so funny. I’d love to write a book with Bob Hard on our time in Andrew’s lab and title it “The Mitotic Pole”.



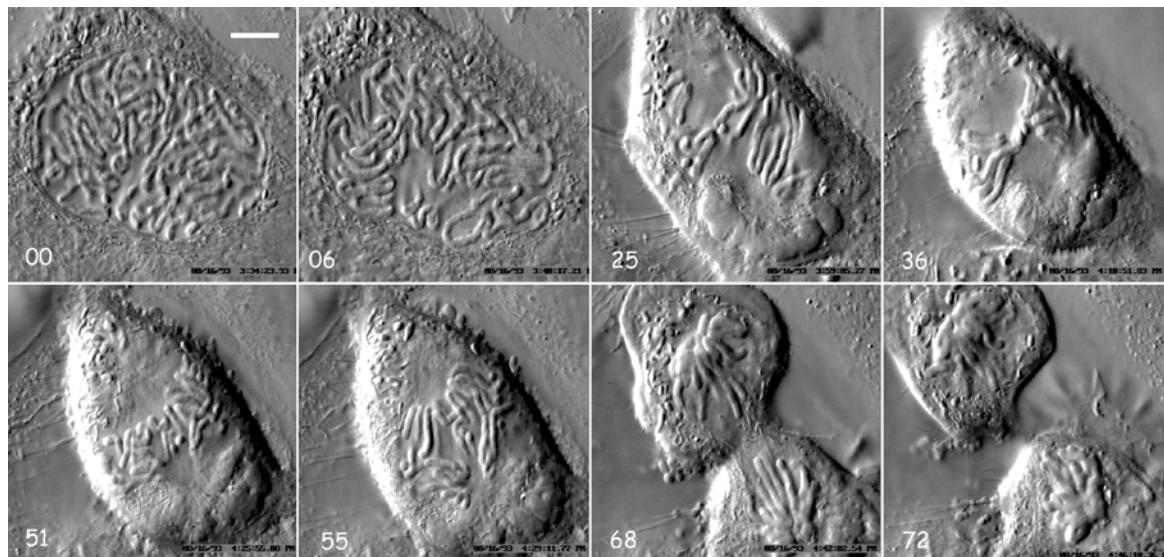
The Northwestern rough skinned newt, *Taricha granulosa*, was abundant in the lakes and forests of Oregon. Cultures from its lung tissues produce extremely flat, large (250- μm diameter), optically clear cells that have many advantages for studying mitosis.

(fig.4)

Andrew had many cryptic phrases of wisdom including the statement, uttered in response to my showing him new data, that “even a blind chicken can sometimes find a piece of corn if you put it in front of his nose”. His favourite saying, which I still don’t fully understand, was that “the cell is always speaking; the secret is to learn its language”. Frequently he generated what he thought were exciting ideas that Hard and I usually ended up wasting time on. One Monday when we showed up for work Andrew led us over to two huge plastic ice coolers each of which was about 4 feet long, 3 feet wide and 3 feet high. He was really excited as he lifted the lids off both to reveal two rare, giant white sturgeon heads, from 100+ year old 1000 lb fish that he had caught in the Columbia River that weekend with a research permit. This was several years after Richard Weisenberg had shown that brains were the best source of tubulin for *in-vitro* microtubule re-assembly studies. At the time others were using cow or pig brains to isolate tubulin because they were readily available from local slaughter houses. Andrew thought it would be interesting to study tubulin that came from an ancient creature that had changed very little over their 175 million year history. His orders to us that morning were to remove the brains which we thought would contain a 20 year supply of tubulin. Many hours later, after much dissection and a rushed literature search, Hard and I discovered that despite their enormous size, the brains of these ancient fish consisted of half a dozen or so thumbnail size neuronal ganglia scattered throughout the volume of their cranium. We had to abort the project. The only salvageable aspect of the whole fiasco was that Andrew now had a lifetime supply of meat for his dogs.

My graduate routine was to show up in the lab sometime before noon, try to avoid Andrew so I wouldn’t have to type anything, and film newt cells well into the night. I would record for about a month and then send the film off to be developed in a Hollywood studio. When it came back I would splice together the parts that I wanted to make a copy of – because you never ever worked from the original negative – and then send the negative off again so that a positive could be made for analysis. So it would be 3 months later before I could see what I had. By the time 3 years had gone by I had a pretty extensive library of films of mitosis. And of course I was looking at all kinds of behaviours, first in real-time and then in time-lapse, from nuclear envelope

breakdown and chromosome mono-orientation, to chromosome congression, chromatid separation, anaphase, telophase, and cytokinesis. During this time I fell in love with mitosis.



(fig. 5)

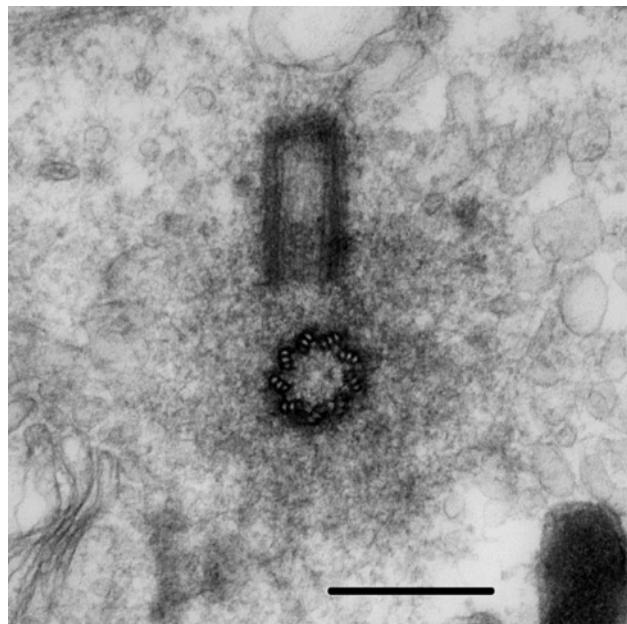
This figure was constructed from individual frames of a time-lapse DIC video light microscopy series of mitosis in a newt lung cell, filmed on August 16, 1993. In the second frame the nuclear envelope has just broken down to initiate prometaphase, the stage of spindle assembly. The cell is in metaphase with a fully formed spindle and congressed chromosomes in the upper right hand frame, and has entered anaphase in the bottom left hand frame. It then undergoes telophase and cytokinesis in the last two frames. Newts are cold blooded and their cells grow best around room temperature. At 21°C they take anywhere from 1-8 hours to complete a division, depending on their degree of flatness. Time in minutes is relative to the first (00) frame. Scale bar in 00= 10 micrometers

How did you decide where to go and who to work with for during your post-doctoral years and how did that lead on to your first independent position?

By the time I graduated from Andrew's lab he and I had established a mutual respect for one another and, in part to show his appreciation he lined me up with two post-doctoral offers from people I had never met. One was with R.D. (Bob) Allen at Dartmouth College who was pioneering high resolution Differential Interference Contrast Light Microscopy (DIC LM), and who later was involved in the discovery of video-enhanced LM. The other was Hans Ris who was developing high voltage electron microscopy (HVEM) at the University of Wisconsin. Both had terrific reputations—Allen was one of the founders of the field of cell motility while Hans was a National Academy member who had used LM and stains to show that chloroplasts contained DNA and were likely endosymbionts. Both had published several papers on mitosis. I had had a pretty large dose of LM in Andrew's lab so in May of 1977 my girlfriend (Susan Nowogrodzki and later my wife) and I moved to Madison to work on the structure of microtubule organizing centres. I then spent 2 years defining the high resolution 3-D ultrastructure of various microtubule nucleating organelles, publishing 3 solo author and 3 collaborative papers none of which had Hans's name on them. Hans was already a well-respected scientist and an extremely unselfish mentor, and he wanted me to be credited with my own work. At the beginning of my 3rd year my position with Hans expired and I moved to Gary Borisy's lab so I could complete a time-consuming same-cell correlative LM/HVEM study on kinetochore fibre formation in mammals. The result was that by the end of 1979, I had a number of first author papers and had established myself as one of only a handful of experts in the emerging field of biological HVEM.

After three years in Madison I began to search for a position that would pay me more than \$11,500 per year. My only other criterion was that it was as an independent researcher and not as an EM technician. I was fortunate in that several years earlier Donald Parsons of the Roswell Park Cancer Institute had convinced David Axelrod, who was then the New York State Commissioner of Health, to buy a 1.2 MeV HVEM which was to be housed in a new Public Health Laboratory within the newly constructed Empire State Plaza in downtown Albany. From 1975-1979 Parsons recruited a number of highly talented individuals to work on extracting the

maximum information possible from the HVEM. These included Joachim Frank, Carmen Mannella, Tony Ratkowski, James Turner and Mike Marko---- all of whom were mathematicians, physicists, or biophysicists. However, by late 1979 it was evident that NIH/NCRR, which supported the HVEMs run by Ris in Madison and Keith Porter in Boulder as National Biotechnological Resources, was not going to fund the Albany HVEM because it lacked a cell biologist. I was then recruited to fill this void. I have been in the same lab space 50 feet underground ever since and our HVEM is still functional 32 years later.



(fig.6)

High voltage electron micrograph, from a 0.25 μm thick section, through a centrosome found on the surface of a telophase nucleus in a PtK1 cell. This beautiful organelle consists of a mother and daughter centriole pair surrounded by a less electron dense “cloud” of pericentriolar material. During mitosis centrosomes nucleate radial (astral) arrays of microtubules involved in spindle assembly. Scale bar = 0.5 micrometer

Throughout your career you seem to have stayed ahead of advancing technology and used your skills to work on problems with a high tech approach. How have you been able to do this?

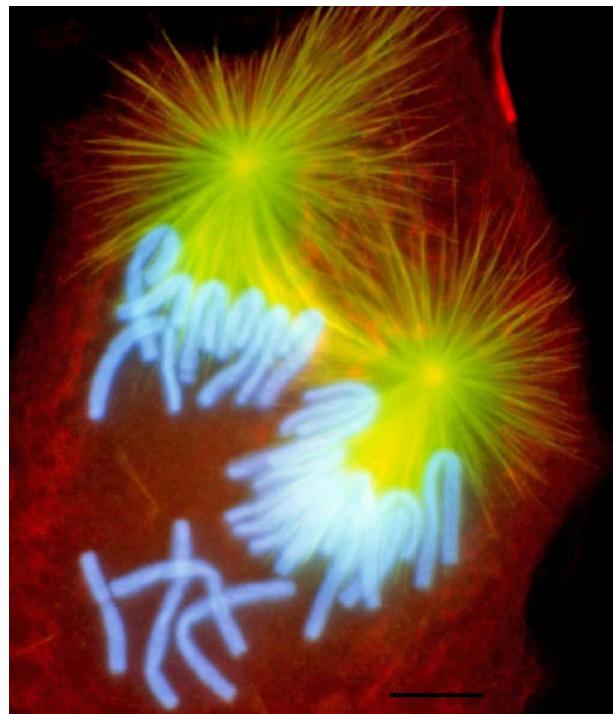
I've been working with big and expensive equipment since I was a post-doc, after which my affair with high technology continued primarily because I was in the right places at the right times. I was fortunate when I took my job at the Wadsworth Centre in Albany in that N.Y.S. funding allowed me to set up my lab with extremely specialized equipment and to hire extremely proficient technicians. Also, during that first year in Albany I spent several summer days at the Marine Biology Laboratory (MBL) in Woods Hole where I met Ted Salmon and Greenfield (Kip) Sluder—both of whom were ably trained in LM by Shinya Inoue.



Edward (Ted) Salmon and Greenfield (Kip) Sluder during a “block” party at the Rieder MBL Memorial Circle cabin on August 12, 2009.

(fig.7)

I realized, right away, that Woods Hole was a fulcrum of scientific activity and thought, and it was also the ideal place to escape the heat of Albany during the summer. It has great beaches, my wife and kids would love it, and I could schmooze as much as I wanted with scientists from all over the world. As a result the next year I persuaded the N.Y.S Commissioner of Health and the Director of the Wadsworth Centre to allow me to spend my summers at Woods Hole. This quickly led to collaborations with Kip on using the HVEM to answer questions about centriole replication in sea urchin and starfish zygotes. These were incredibly time consuming studies in which Kip used polarization LM to follow individual fertilized sea urchin zygotes to a critical point, after which he fixed and embedded them. At this point I took over. To make such studies even feasible I had to develop some novel approaches which involved using LM to pre-screen the content of serial sections cut for HVEM. I'm sure I'm the only one that has ever serially sectioned one, yet alone many, whole sea-urchin zygotes looking for centrioles—basically two needles in a haystack.



(fig.8)

An indirect immunofluorescence image of a newt lung cell in prometaphase of mitosis after staining for microtubules (yellow), chromosomes (blue) and keratin (red). As the nuclear envelope broke down in this flat cell several chromosomes (bottom left hand corner) came to lie away from the centrosomes and forming spindle. Live cell video-enhanced light microscopic studies on such “lost chromosomes” proved in the late 1980’s that during spindle assembly microtubules that form the kinetochore fibers are derived from aster microtubules growing from the centrosomes. Scale bar = 10 micrometers

While in residence at the MBL in 1980 Inoue and R.D. Allen discovered video-enhanced LM which led directly to Ron Vale’s discovery several years later, also at the MBL, of the first non-axonemal microtubule motor protein (kinesin). The development of video LM changed the whole ball game for me—and with Teds help I established the necessary technology in my Wadsworth lab in the mid 1980’s. I then began to work in earnest on defining the behaviour of microtubules, centrosomes and kinetochores at high spatial and temporal resolution. My days of waiting months for my 16-mm films to be processed were over because I could now view events in time-lapse or real time immediately after recording them.

In the spring of 1985 Ted and I travelled to UC Irvine to visit the NIH/NCRR Biotechnological Laser Microbeam Resource that had been established by Michael Berns, who pioneered the field of modern laser microsurgery. We were trying to determine the potential of the system for surgical studies on dividing newt cells. I think it was the first day when we discovered the “polar winds” which ejected chromosome fragments from the centrosome (spindle pole) regions. Mike’s system was configured so that the shutter and stage movements were controlled by a single joy-stick, and all operations took place in the dark with nanosecond pulses of green light obtained by frequency doubling the rather loud output of a neodymium:yttrium-aluminum-garnet laser. It was like playing shooting games in an arcade---complete with sound effects--and Ted and I had a lot of fun cutting chromosomes and following their subsequent behaviour. Over the next few years we returned periodically to Mike’s lab to conduct other studies.

In early 1989 Lee Hartwell, who was working on his cell-cycle checkpoint concept, phoned to ask if there was any evidence that cells actually waited for all chromosomes to move to the spindle equator before initiating anaphase. I was working on kinetochore function and chromosome congression at the time, and was aware from my thesis days that Raymond Zirkle published an ASCB abstract on this very topic in 1970 (which he never worked up into a full paper). I told Lee that the answer was yes, but that the evidence was mostly anecdotal and the mechanism was completely unknown, and I gave him several references. After hanging up the phone I remember thinking—wow—I bet I could determine using laser microsurgery why anaphase only starts after all chromosomes congress. I put that idea aside and went on to finish what were at the time more-interesting ongoing studies. However, in 1993 I was able to convince the Director of the Wadsworth to purchase all of the many component parts needed to assemble my own DIC based laser microsurgery system, which we constructed in early 1994. As we were assembling the system I drew up a list of cool studies that we could conduct once it was up and running and the kinetochore checkpoint work (which we ultimately published as a series of papers in 1994/1995) was at the top of the list.

I’ve been at the right place at the right time more than once. In May of 1990 I was invited along with Kip Sluder, Gary Borisy (currently the Director of the MBL), Lee Hartwell and about 15 other western scientists to attend a two-week conference in Leningrad, Russia organized in part by Bill Earnshaw who was then at Johns Hopkins. The conference took place during the “white nights”, when the sun never sets, and during this period I got no sleep and lost 20 lbs. There were many highlights, but one was touring the Hermitage with Alexey Khodjakov who at the time was a Ph.D. student in Moscow working on centrosomes. In 1993, after the failed coup attempt in Russia, Alexey wrote to Kip Sluder and me asking if we had any post-doc positions available. Lucky for me I did and Kip did not. Alexey joined my lab in 1994 in time to help clean up the kinetochore checkpoint work, and to initiate some of the other studies on my list. Then in late December of 1996 he came to me with the idea of using Green Fluorescence Protein (GFP), which had just been shown by Martin Chalfie to work as a fluorescence reporter *in vivo*, to selectively tag kinetochores and centrosomes. This was a terrific idea because by selectively lighting up an organelle it would eliminate a major ongoing problem which was the uncertainty in positioning sub-resolutional non-membrane bound structures during the ablation part of the microsurgery process. After re-configuring the laser system for fluorescence LM we published a proof of concept paper which we used as the basis for several experimental papers on centrosomes. Since then Alexey has continued independently to develop the approach to the point where he can now selectively remove a budding pro-centriole from the wall of its mother without damaging the original centriole.

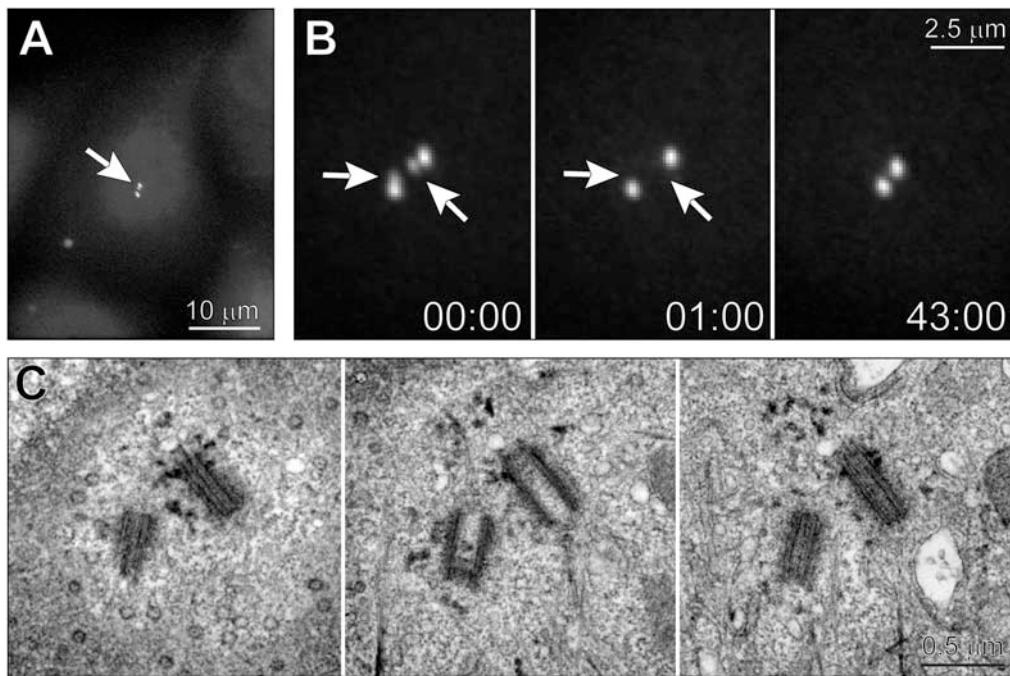


Fig. 9. Daughter centrioles can be ablated as they form in a replicating centrosome. (A) A HeLa (human epithelial) cell during S phase of the cell cycle. The centrosome (arrows), which is composed of two centrioles, is labeled via centrin/GFP expression. (B) A higher-magnification view of the centrosome reveals that both mother centrioles have already developed short daughters (arrows). Both daughter centrioles were irradiated (*cf.* arrows in 00:00 and 00:01) with short series of laser pulses (~10 per centriole), and 43 min later the cell was fixed for an EM analysis. (C) Serial-section EM revealed that both daughter centrioles were completely ablated while mother centrioles remained structurally intact. Time is in minutes: seconds. Reprinted from Methods in Cell Biology, Vol 82, Valentin Magidson, Jadranka Lončarek, Polla Hergert, Conly L. Rieder, Alexey Khodjakov, Laser microsurgery in the GFP-era—A cell biologist's perspective, 239-266, 2007, with permission from Elsevier.

So now it was the science motivating technology development.

Actually, developing a particular technology to solve a specific set of problems has been at the core of my efforts since joining the Wadsworth. From 1980 to 2000 I was a key player in, and for 10 years the principal investigator of, a NIH/NCRR funded National Biotechnological Imaging Resource grant. The primary goal of NCRR funded Resources is to develop a particular technology or set of technologies in response to a proven driving need by the biomedical user community. As an example, although an HVEM can image all of the biology within a 1-2- μM thick section, it puts it all onto a single 2-D screen or negative—and it is impossible to extract 3-D information from single 2-D images. Early attempts at overcoming this problem, including stereo viewing of tilted images and specific staining, proved of limited value because they produced low resolution images applicable to a small set of problems. For most studies it remained impossible to extract the desired high resolution 3-D information contained within the section volume. To overcome this problem Joachim Frank, Bruce McEwen, Jim Turner, Mike Marko and I spent many years developing HVEM tomographic approaches for extracting useful high resolution 3D EM information from thick sections. One of the more notable papers that came from this effort was the first tomographic reconstruction of the mammalian kinetochore—an organelle that I had been working on since graduate school. Many scientists, including Mike and Bruce, still work on perfecting EM tomography which through the years has developed into a very powerful and useful technology.

Since graduate school the focus of my work has been on motile phenomena of which mitosis is just one example. Early on I would seek collaborative projects on an aspect of motility that I could solve with the equipment and methodologies developed in my lab—but this was only after we brought the technology to the point with our own questions where it was of proven value. So during the first 20 years of my career I published papers on 10 or so different organisms—from various fungi, protists and strange flies that live in wasps, to sea urchins, starfish, newts, rat-kangaroo's and humans.

Of course one problem of relying on cutting edge technology is the incredible expense. Take the development and implementation of video-LM in the 1980's for example. This awesome approach allowed us to shoot lengthy real-time or time lapse sequences and then play them back instantly, instead of having to send them off to the photolab and wait weeks for the result. Originally the images were stored on VHS video tape which was quickly supplanted by optical memory disk recorders and then by computer hard drives. During a three year period in the late 1980's we went through 3 optical memory disk recorders at around \$35,000 each. We'd buy one and a year later a new, improved format would come out and we'd buy that one and the old one would suddenly become a door-stop (obsolete) because it was no longer supported. Later we had to buy various types of disk readers just to be able to maintain access to our data. It became evident early on that I needed a constant influx of around \$200K a year to maintain my equipment base, and that hasn't changed.

Could the work that you have been doing recently been done in the 70s?

Work on mitosis during the 1970's consisted primarily of LM descriptions of how chromosomes and other spindle components behaved in living cells, or what the various components look like at the EM level—even in yeast!. During that period, methods for identifying the molecules involved in mitosis were hit and miss and were limited to indirect immunofluorescence LM on dead cells. In the 1970's biochemical studies on dividing cells were crude and focussed exclusively on lysed cell models which never faithfully recapitulated the process under study—and which told us more or less what we already knew from live cells. The breakthrough in this area did not come until the perfection of *Xenopus* oocyte extracts for spindle assembly in the mid to late 1980s. This, combined with information derived from the various genome projects, allowed one to determine whether a given protein was critical for spindle assembly by simply immunodepleting it from the egg extract. When I was in graduate school I was absolutely convinced that the molecular basis for the “anaphase trigger”, the mechanism by which chromatids separate to initiate anaphase, would not be solved in my lifetime. This would have been true had I died in 1992, before Andrew Murray and others cracked the problem using *Xenopus* extracts and yeast genetics.

The focus of my recent work is still on understanding the molecular basis for behaviours exhibited by dividing cells. Currently I'm working on how cells slip through mitosis when they cannot satisfy the mitotic checkpoint and why cancer, but not normal, cells die during mitosis in response to taxol. While the behaviours (slippage and death during mitosis) could have easily been defined with the time-lapse techniques available in the 1970's, none of the current tools were available at the time to even start to understand their molecular basis.

What, in your opinion, does microscopy still have to offer in relation to progress in understanding cell division?

Well, the days of publishing meaningful non-molecular based LM or EM observations on a particular cell, process or behaviour are over. However, LM will continue to play a key role in defining the function of the many novel proteins cloned from the various genome projects, because it forms the basis for most primary screens for mutants or phenotypes associated with inhibiting, deleting or knocking down specific proteins. So microscopy is and will continue to be an important tool in defining which proteins are involved in mitosis, how their location changes during the process, and which of the many sub-processes that comprise mitosis these proteins are involved in.

That being said, there is also an enormous amount of developmental work going on at the forefront of imaging with light. This includes hardware and software developments as well as specimen preparation procedures. Mitosis is comprised of a series of behaviours that need to be defined mechanistically at the molecular level. These behaviours can only be discovered and characterized by live cell imaging; and the more detailed the temporal and spatial characterization of a particular behaviour, the more accurate the resulting molecular model will be. We are now able to tag multiple proteins with different fluorophores without disrupting their function, so that they can be followed in 4-D with high temporal and spatial resolution for long periods without damaging the cell. It is a stunning Nobel-prize winning technology that has already forced a radical redefinition of how kinetochores behave. I predict that it will continue to evolve and will remain the most powerful approach for elucidating the mechanics of mitosis. State-of-the-art live cell imaging changes at warp speed and there is no doubt that the advances made in the future will keep young investigators busy on mitosis for generations to come. Most, if not all, of the more obvious behaviours exhibited by centrosomes, kinetochores and chromosomes during mitosis have been discovered. However, how the various proteins

involved in mitosis interact and change position over time, and what this means, remain to be determined. These questions are approachable with Total Internal Reflection Fluorescence microscopy (TIRF), Fluorescence Resonant Energy Transfer microscopy (FRET) and other LM techniques under development. It's worth noting that the molecular mechanism for correcting errors in kinetochore attachment, which I think is the most recent spectacular finding on mitosis, was discovered through a combination of yeast genetics and live cell 4-D imaging.

I feel less enthusiastic about the future contribution of EM to mitosis research. I think it will be limited primarily to defining the high resolution structure of those molecules or macromolecular complexes involved in the process, that cannot be crystallized but that can be isolated in pure form. I say this because there are critical and difficult specimen preparation problems that need to be solved before EM can be used on sections of cells to more than simply confirm what is suspected from high resolution LM studies. The major problem is to know exactly where the proteins that you are interested in are in the EM image. Rapid developments in super resolution LM have largely supplanted the older EM immune-gold staining methods, which for the most part worked only with fixation protocols that destroyed high resolution structural relationships. Members of the Wadsworth and others are working on methods to mill frozen cells into slabs containing a specific area of interest, for 3-D vitreous tomography—a technique that could, in principle, provide a minimally distorted snapshot of where every molecule was at the time of freezing. However, for this to become useful, methods must be developed for identifying the molecules of interest within the images, i.e., generating contrast throughout the thickness of the specimen specifically for the protein(s) under investigation—since most proteins in non-fixed, non-stained vitreous thick samples are invisible in the EM. So, there is now an effort to figure out how to solve this issue, but I'm not optimistic that it will be solved in the near future.

What do more unusual systems have to tell us about mitosis (and meiosis) that many of today's investigators are missing, either because they simply don't know about them or they can't be bothered, or they are unfashionable? Are we too hooked on "model systems" that might seem more promising when looking for a cure for cancer and more appealing to funding agencies? I guess I'm thinking mainly about all those amazing things that were described by Wilson 100 years ago, many of them in insects.

The answer to this question depends on how you define mitosis and what you want to know about the process. Let's focus for a minute on mitosis in humans and define it to include all of the events that occur during the cell cycle—because clearly what happens during mitosis is predicated on earlier events. In this case, our understanding of how human cells are driven into an out of mitosis is based primarily on discoveries in yeast, frogs and sea urchins. Much of what we know about how centrioles/centrosomes replicate in human cells came from studies in the worm and fly. Most of the kinases that drive spindle assembly and error correction in human cells, like auroa and polo, were discovered in flies. Finally, what we know about chromatid separation and exit from mitosis (telophase) in human cells has been garnered primarily from work on yeast and frog oocytes. I am unaware of any major discovery about mitosis in humans that has been made in human cells. Rather a handful of diverse model systems have contributed to how we currently understand the division process. Although some workers continue to use unusual "orphan" systems to study mitosis and meiosis, the number is dwindling. There are several reasons for this. First, for such systems to be generally useful their genomes must be sequenced. Second, they must also offer a clear advantage over existing proven systems like yeast, worms and flies that can be easily genetically manipulated.

We now know that if you remove the centrosomes from fly or human cells they form functional bipolar spindles, and if you knock out the cytoplasmic dynein motor protein at the same time, they form spindles that look like the ones formed by plants, leading to the inescapable conclusion that there are multiple "redundant" mechanism for spindle assembly in animals: animal spindles really are just plant spindles with centrosomes and dynein. It is also clear that there are at least two mechanisms for generating the forces to move a chromosome: one is based in the kinetochore and the other along the kinetochore fiber or in the pole. Some cells (like crane fly spermatocytes and *Xenopus* oocytes) use primarily just one mechanism while vertebrate somatic cells use both to different degrees. When I was a student the dogma was that because an error free mitosis is so vital to all organisms, the underlying force producing mechanism (singular) that leads to chromosome segregation must therefore be highly conserved. As a result, conclusions from one system were automatically accepted as true for other systems, which in retrospect led to much confusion. Try as we did, for many years it was difficult to understand the meaning of Art Forer's neat Ph.D. thesis observation that in crane fly spermatocytes holes in kinetochore fibers created by a UV microbeam moved rapidly poleward; similar holes created in vertebrate somatic cells did not appear to move. Little did we know at the time that in addition to a mechanism being "highly conserved", fidelity can also be ensured by redundant mechanisms working to lesser or greater degrees at the same time.

I view the morphological and behavioural variations in mitosis and meiosis, so carefully described by E.B. Wilson and later by Frans Schrader and his wife Sally-Hughes Schrader, to be largely manifestations of emphasizing one mechanism for forming a spindle or moving a chromosome over another. The real issue is what do you choose as a reference point to compare these variations against? For those of us who require a healthy, constant renewable supply of funds for our labs, the reference must be the human system or one that has been shown by past work to be directly relevant to humans. That being said, there is a real tangible advantage in knowing the early literature and the behavioural diversity exhibited by different cells and organisms during mitosis/meiosis. A terrific example of this is Bruce Nicklas's very cool 1995 Nature paper in which he showed that putting tension on the last kinetochore to attach to the spindle (by pulling on the chromosome) allows the mitotic checkpoint to be satisfied. The overlooked key to this study is that Bruce did it in mantid (*Tenodera aridifolia sinensis*) spermatocytes containing a non-natural univalent chromosome. Due to the error correction mechanism, these univalent chromosomes would never establish a stable connection to a spindle pole unless he stabilized the connection by pulling on the chromosome. Few know that Bruce worked with the Schrader's at Columbia in the late 1950's and was well aware not only that non-natural univalent chromosomes led to an arrest in meiosis, but also that they were prevalent in the mantid, an easily available organism in North Carolina. Now here is an individual who spent 45 years working on grasshopper spermatocytes, who because of his knowledge of the literature realized in 1994 that there was a natural solution to one of the major questions in the field at that time. The resulting paper was his only publication ever on the mantid! I seriously doubt that the reviewers asked him to confirm his findings in a more "acceptable" system before publication. The reason for this being that the key proteins for the mitotic checkpoint were discovered several years earlier in yeast and shown to be highly conserved. The fact that he answered the question in a meiotic system and not a mitotic one was irrelevant, because it is now generally accepted from experimental evidence that meiosis is simply two back to back mitoses, the first of which is modified by the bivalent character of the chromosomes. It's interesting that Bruce's paper contains no gels or biochemistry, but was based simply on live cell imaging, a microneedle and some indirect immunofluorescence studies!

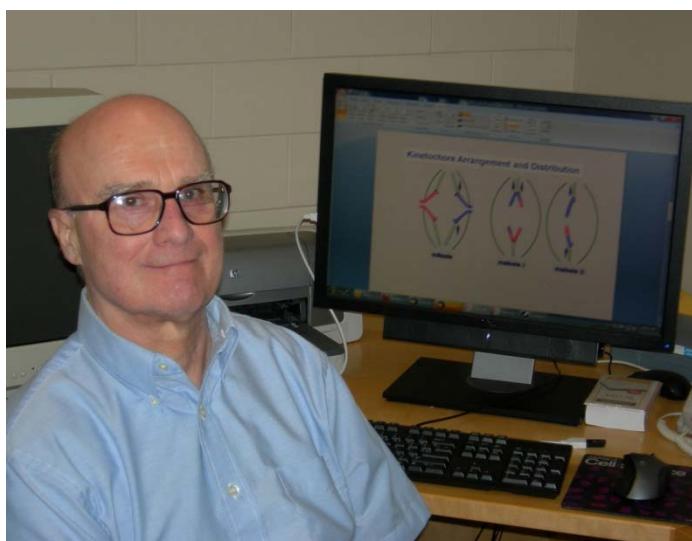


Figure 10

Bruce Nicklas 2010

As exemplified by the Nicklas study, the diversity of spindle structure and chromosome behaviours exhibited during mitosis and meiosis can at times prove useful in problem solving. However their utility is predicated on first knowing the unique attributes of various organisms (like Bruce did) and these days few students read or study the older literature. Also, in my view, hypothesis-driven experimentation, which was mandatory for funding in the 1980's to 1990's, is being replaced by cook-book and largely automated isolations, purifications and rote characterizations of genes and their function. Since the unusual divisions you refer to are mostly interesting because they provide "natural" experiments, and since experimentation in biomedical research is no longer in vogue, there is little reason for students to study the diversity of mitoses except to satisfy their curiosity. Again, in my view, most students either don't have the time or the inquisitive mind for this literary research.

Theodosius Dobzhansky once said “Nothing makes sense in biology except in the light of evolution”. Would it, in your view, be possible to construct the evolutionary story of mitosis – how it all began and how it evolved into the highly sophisticated process that we see in higher organisms today or have attempts already been made to do this and not proved especially rewarding?

This question is related to the previous one. Several attempts have been made to construct an evolutionary story of mitosis but there are too many missing links that doom such projects from the start. Evolution is clearly responsible for the redundant mechanisms seen in human cells for forming a spindle and moving chromosomes--any change that introduces a novel backup system that enhances the fidelity of mitosis will be selected for. However, the discovery of these redundant mechanisms did not come about through studying how the spindle evolved, but rather via experimentation on insect and vertebrate cells. Unfortunately, because it has little demonstrable impact on the public who pays the research bills, when and how these various mechanisms arose in evolutionary history have been relegated to interesting philosophical questions. As a result few in major research institutions work on the evolution of mitosis because their job, promotions and salary are directly linked to the amount of grant money they generate. Instead such issues are explored on shoe-string budgets, often in small liberal art colleges where such research forms a valuable part of the academic experience because of the skills it teaches. In my opinion rather than set out to construct the evolutionary story of mitosis from the literature or even new research, it would be better and much cheaper to simply wait for it to construct itself—because that's slowly happening.

What were the most exciting moments of your career?

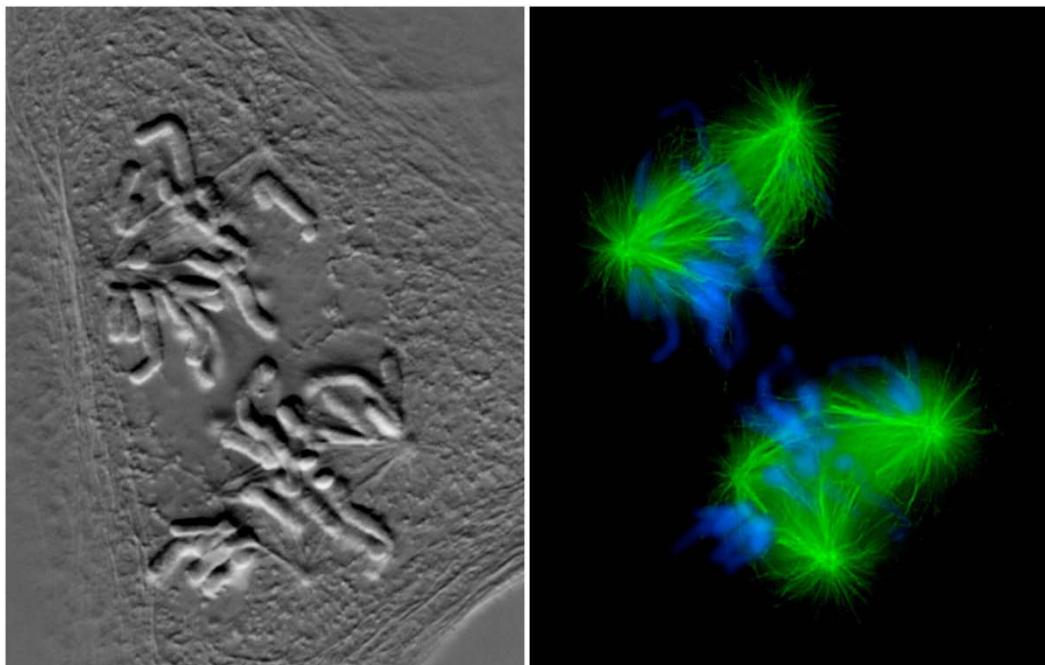


Fig. 11. Cell fusion methods were used to create a rat kangaroo (PtK1) cell that contained two independent mitotic spindles. Live cell observations on such cells revealed that “wait anaphase” signal produced by unattached kinetochores is not diffusible and that its target is in or near the spindle containing the unattached kinetochores.

By far the most exciting times were those few short inspirational periods when I realized that the experiment I just thought up was not only doable but also a “win-win” situation, meaning that regardless of the outcome the result would be important and publishable in a top journal. As an example, after we found that unattached kinetochores released a “wait anaphase” signal I wondered whether this inhibitor was diffusible, or if it targeted something associated with the spindle. As I was pondering this question it dawned on me that the perfect way to discriminate between these possibilities would be to create a cell containing two independent spindles adjacent to each other, one of which possessed one or more unattached kinetochores at a time when the other had already satisfied the mitotic checkpoint. If the unattached kinetochores on one spindle inhibited anaphase in the neighbouring spindle that lacked an unattached kinetochore, then the inhibitor was diffusible.

On the other hand, if the spindle lacking unattached kinetochores entered anaphase in the presence of a neighbour containing one or more unattached kinetochores, then the inhibitor was not diffusible and its target was near or in the spindle containing the unattached kinetochores.

We used cell fusion to do the experiment and the result was clear—the wait anaphase signal generated by an unattached kinetochore is not diffusible and targets something associated with the spindle containing the unattached kinetochore. This experiment also showed that the “go anaphase” signal, generated after the mitotic checkpoint is satisfied, is diffusible and can force a spindle into anaphase even if it contains unattached kinetochores. Although this was a very satisfying experiment, it was too far ahead of its time as is evident from the fact that the 1997 paper reporting the result has been cited more in the past few years than in the years immediately after it came out.

There are now a bewildering number of molecules, all with their own special acronym that seem to be involved in regulating the various integrated processes involved in the mitotic cell cycle. Do you think the day will come when we will be able to put the jigsaw together and present a comprehensive description of the entire process, G1 through M, with a full understanding of the roles of each of the molecules involved?

No!

In the headlong drive for reductionism and against the background of the enormous amount that is now known about mitosis, what questions remain? What's left for the next generation of young-uns to get their teeth into and forge their reputations on?

If I knew the answer to this question I wouldn't retire!

Looking back on your life up to the time when you left Oregon, can you identify any transferrable skills, not in any way related to biology, that you were able to exploit in later years as an experimental biologist?

When I was 12, I became a serious coin collector which involved spending hours looking at coin surfaces with a magnifying glass. This led to a keen appreciation and interest in minute details, which continues to this day. Later, due to family issues, I was forced to work full time while I attended undergraduate school. This taught me time-management skills that have proven extremely useful throughout my life. In a way this experience made me a bit impatient, so if a project we were working on did not show promise after 3 months, I simply bagged it and started a new one. I'm not much for banging my head against a wall—and that came from developing a real value for time when I was younger. Finally, during the last two years as an undergraduate I worked in a medical library, basically doing literature searches for MDs. This led to a keen appreciation

for the history of a particular scientific problem, which as a graduate student led me to digest all I could on what was written about mitosis. I believe it fair to say that my colleagues still consider me to have an outstanding command of the early literature—at least up to about 1990 when it started to get out of hand.

What advice would you offer to those just entering the field?

I tell most students to ask themselves “What do I want to be doing 5 years from now?”, and then work to make it happen because the time will go quickly! The consensus among my peers is that those who work 60-hr instead of 40-hr weeks as a graduate students and post-doc will ultimately get the better job—as long as they are using the time wisely. That being said the best advice I can give to someone interested in researching mitosis is to become wedded to the entire process and not to just a single molecule or avenue. That way you can rapidly change your plan of attack, without an inordinate amount of down time, if the avenue you are working on gets too crowded, suddenly comes to a dead end, or simply becomes boring. At appropriate times in your career you can also launch attacks along multiple avenues and then focus on those that look most promising. I also think it beneficial to find a cool cutting edge technology that you are interested in and learn it well. It may be that it is your experience with this technology, which every department wants and must have, that gets your foot in the door and not the actual biological problem you are working on.

Finally, there is an increasing tendency to declare in a paper title a sexy conclusion that is supported entirely by indirect data—because such titles generate excitement and help with funding. However, they also start new avenues of research that all too frequently turn out to be dead ends, because the original conclusion(s) were erroneous from the start. Therefore be very critical when reading the literature and be especially critical of conclusions based entirely on indirect data like cell-sorting-based population studies and/or indirect immunofluorescence. Along these same lines, I've noticed an increasing tendency to not thoroughly do the appropriate homework before starting a project. Something is wrong with the claim that protein X is required for a functional mitotic checkpoint, when it is clear from earlier literature that knocking protein X out of mice has no phenotype while knocking bona-fide mitotic checkpoint proteins out is lethal at the embryonic stage.

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