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2011 ASCB Award Essays and Selected Feature Articles

From the November 1, 2011, Issue

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The Philosophy of Molecular Biology of the Cell

Molecular Biology of the Cell (MBoC) is published by the nonprofit American Society for Cell Biology (ASCB) and is free from commercial oversight and influence. We believe that the reporting of science is an integral part of research itself and that scientific journals should be instruments in which scientists are at the controls. Hence, MBoC serves as an instrument of the ASCB membership and as such advocates the interests of both contributors and readers through fair, prompt, and thorough review coupled with responsible editorial adjudication and thoughtful suggestions for revision and clarification.

Our most essential review criterion is that the work significantly advances our knowledge and/or provides new concepts or approaches that extend our understanding. At MBoC, active working scientists—true peers of the contributors—render every editorial decision.

The Society and MBoC are committed to promoting the concept of open access to the scientific literature. MBoC seeks to facilitate communication among scientists by

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MBoC publishes studies presenting conceptual advances of broad interest and significance within all areas of cell biology, genetics, and developmental biology. Studies whose scope bridges several areas of cell and developmental biology are particularly encouraged. MBoC aims to publish papers describing substantial research progress in full: Papers should include all previously unpublished data and methods essential to support the conclusions drawn. MBoC will not, in general, publish papers that are narrow in scope and therefore better suited to more specialized journals, merely confirmatory, preliminary reports of partially completed or incompletely documented research, findings of as yet uncertain significance, or reports simply documenting well-known processes in organisms or cell types not previously studied. Methodological studies will be considered only when some new result of biological significance has been achieved or when introduction of the method will significantly accelerate progress within a field.

Note that MBoC places a premium on research articles that present conceptual advances of wide interest or deep mechanistic understanding of important cellular processes. As such, articles dealing principally with describing behavior or modification of specific transcription factors, or analysis of the promoter elements through which they interact, will not generally be considered unless accompanied by information supporting in vivo relevance or broad significance.
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MBoC 2011: same values, improved feng shui

Douglas R. Kellogg and David G. Drubin

INTRODUCTION
Molecular Biology of the Cell (MBoC) was conceived nearly 20 years ago as a journal that would be run by and for cell biologists under the auspices of the American Society for Cell Biology (ASCB). The goal was to provide the cell biology community with a journal that would enhance scientific communication among cell biologists by providing authors with rapid, rigorous, constructive, and fair peer review, as well as editorial decisions that were not constrained by journal space or perceived trends. Over the years, MBoC has come to epitomize these values and in so doing has become a pillar of the cell biology community. Today, the values remain the same, but as MBoC heads into its twentieth year we are happy to highlight what we are doing to be more essential and relevant than ever.

ASCB ANNUAL MEETING ISSUE AND COVERAGE
This issue is MBoC’s third annual special issue focused on the ASCB Annual Meeting. It includes a collection of fascinating essays by the recipients of the E. B. Wilson Medal, the Keith Porter Award, the Women in Cell Biology Awards, the E. E. Just Award, and the Early Career Life Scientist Award. Together with invited Perspective and Retrospective essays by other prominent cell biologists, these articles provide insight into research careers, education, mentoring, diversity, science advocacy, and how key discoveries were made.

In addition, for the second year in a row, we have invited chairs of the ASCB Annual Meeting Minisymposia to write reviews of their sessions. These will be published early in 2012.

A REVAMPED WEBSITE
In August, MBoC launched a new and improved website (www.molbiolcell.org). In addition to a more contemporary look, better

Douglas R. Kellogg is the Features Editor of Molecular Biology of the Cell. David G. Drubin is Editor-in-Chief.
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Features article that provided advice on when a project will benefit from theoretical approaches (Fletcher, 2011).

With these improvements, we anticipate that MBoC will do even better at serving the ASCB community. As always, we welcome suggestions for further improvements, as well as suggestions for new Features articles. This is your journal, so let us know how MBoC can best serve your needs!

ACKNOWLEDGMENTS

We thank the ASCB Award winners for their excellent contributions to this volume. We also thank Larry Goldstein, Ted Salmon, and Clare Waterman for contributing excellent articles on science policy and on the discovery of fluorescent speckle microscopy. We are grateful to Janet Iwasa for the beautiful cover image, which was elegantly constructed with images from The Cell: An Image Library™ (http://cellimages.ascb.org). Finally, we thank Mark Leader and Eric Baker for their expert guidance and supervision of all things MBoC.

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A CHANCE ENCOUNTER WITH CILIA

Take my interest in primary cilia. Did I intend to study cilia when I started as a postdoctoral fellow with Peter Jackson at Stanford? I did not have a clue what a primary cilium was back in 2003, so the answer would be a categorical no. Back then, I was set on understanding mitotic exit in mammalian cells. Some groundbreaking work had just been published on the role of the Cdc14 phosphatase in triggering exit from mitosis in budding yeast, and the Jackson lab had initiated a molecular analysis of the mammalian Cdc14 orthologues. But while mitotic exit was clearly a fundamental and exciting problem, the mammalian Cdc14 phosphatases simply would not willingly fit in this pathway. Despite repeated efforts and much hard work, I ended up spending the better part of a year going nowhere (at least scientifically). Then, in the summer of 2004, an undergraduate student named Trip Adler came to the Jackson lab, and it became my responsibility to find him a suitable project. For lack of a more creative idea, it was decided that Trip would conduct a yeast two-hybrid screen using the Xenopus Cdc14 orthologues as baits. A typical day for Trip would start with a drive to Santa Cruz at 4 am to surf at dawn, followed by a return to the lab at midday to monitor his yeast plates and set up some cultures. Somewhat miraculously, we completed the two-hybrid screen, and one hit was particularly intriguing. Here was a protein named ALMS1, the product of a gene mutated in the hereditary disease Alström syndrome, which is characterized by obesity, retinal degeneration, cardiomyopathy, and kidney anomalies. Even though I had never been scientifically drawn to the study of human diseases, I could not help becoming fascinated by a gene product whose dysfunction would cause such a constellation of symptoms. Still, nothing was known about the molecular activity of ALMS1 or the etiology of Alström syndrome, so I kept putting along on the (dead-end) Cdc14 track.

Nonetheless, I kept trying to get my hands on every bit of literature published on this curious disease. And one day things clicked. An article from the *Journal of Pediatrics* (Michaud et al., 1996) made a connection between Alström syndrome and Bardet-Biedl syndrome (BBS), based on the overlap in symptom presentation. Most excitingly, a couple of papers on the localization and possible function of BBS proteins in primary cilia had just been published (Ansley et al., 2003; Blacque et al., 2004; Kim et al., 2004), and primary cilia had just been shown to be essential for Hedgehog signaling in mammals (Huangfu et al., 2003). I was hooked and shifted direction immediately, trying to understand what makes this little organelle so special. Here was an entire organelle about which I and nearly all my colleagues knew nothing; it appeared to be essential for processing a variety of signals and had been linked to several fascinating hereditary disorders. The potential for fundamental as well as medically relevant mechanistic advances was enormous. Framing a project and getting our first results still took a considerable amount of time (we published our discovery of the BBSome, a core complex of BBS proteins, in 2007; Nachury et al., 2007), but I was confident I had now found a rich niche and all the hard work would pay off. I am particularly grateful to the trust given by my collaborators for allowing me to pursue this line of inquiry.

ABSTRACT How did I get to become a cell biologist? Or, more generally, why do things happen the way they do? The answer provided by the philosopher Democritus and later adopted by Jacques Monod is “everything existing in the universe is the fruit of chance and necessity.” While I read Monod’s book *Chance and Necessity* as an undergraduate student, little did I appreciate the accuracy of this citation and how much of my scientific trajectory would be guided by chance.
advisor Peter Jackson, who gave me the freedom to radically change scientific projects during the course of my postdoctoral work. In hindsight, the odds of tracing such a path from mitotic exit to primary cilia seem astronomically low, and such a trajectory could never have been planned. Thus, for the most part, it is chance that presented me with a novel direction. But necessity was also a key ingredient: studying the role of Cdc14A and B in mitotic exit was unlikely to yield an important publication, even less a faculty position. I was ready for almost any alternative opportunity, and when something exciting materialized, nothing was there to hold me back.

A CHANCE MOVE TO THE HOME OF MITOTIC SPINDLES

Next, take my work on mitotic spindle assembly. Did I intend to work on mitosis or microtubules when starting graduate school? Not for a single second. I joined the lab of Karsten Weis (winner of the 2006 ASCB Early Career Life Scientist Award) with the sole intention of studying the role of the GTPase Ran in nucleocytoplasmic transport (Nachury and Weis, 1999). Yet, at the very same time that Karsten decided to move his lab from the University of California, San Francisco, to Berkeley, where his lab was next to the lab of Rebecca Heald (winner of the 2005 ASCB Women in Cell Biology Junior Award), a string of papers were published that demonstrated that Ran itself functioned in mitotic spindle assembly, the central theme of Rebecca’s laboratory. How could the timing of Karsten’s move have fitted so perfectly with the link between his research and Rebecca’s? Pure unadulterated chance, and a phenomenal opportunity for someone halfway through graduate school to work on one of the most exciting mechanistic problems in cell biology at the time. Figuring out how Ran regulates spindle assembly required some creative approaches to dissect the molecular intermediates between Ran and microtubules. I benefited from the joint expertise of the Weis and Heald labs, which showed me how the whole of a collaborative group is much greater than the sum of the individual parts. Depleting mitotic extracts with Ran itself and subsequently assaying spindle assembly (Nachury et al., 2001) seemed like the most natural experiment to do, yet it is highly unlikely such an experiment would have been done if the Weis and Heald labs had not been so tightly connected.

CHANCE YIELDS A TRANSFORMATIVE HYPOTHESIS

Finally, take our recent discovery of the tubulin acetyltransferase (Shida et al., 2010). My previous studies of the BBSome had primed us to consider a role for the BBSome in tubulin acetylation (Loktev et al., 2008). But how were we to identify the tubulin acetyltransferase? It turned out that the purification of BBSome-associated proteins from retina recovered one single entity, an uncharacterized gene product named C6Orf134. No domains were to be found in C6Orf134, and this BBSome-associated protein was quickly destined for oblivion. Without much hope, I contacted my close collaborator Fernando Bazan (then at Genentech), a wizard at uncovering distant similarities between proteins using secondary structure–guided alignments. Within 5 min, Fernando emailed back that C6Orf134 looked very much like Gcn5, the prototypical histone acetyltransferase! We have since then validated C6Orf134 as the tubulin acetyltransferase, but chance played a major role in the generation of the hypothesis. If I had not known Fernando, none of this work would ever have been initiated.

FINAAL THINKS

1. Don’t be afraid to fail. Failure is part of the scientific progression. Most importantly, failure is a temporary condition (“giving up is what makes it permanent,” in the words of Marilyn vos Savant). Had the project on Cdc14 worked, I would not have been open to explore a fundamentally new direction.

2. Cultivate scientific relationships. “My” best ideas have come from open discussions with some phenomenal colleagues. So these ideas are not really mine, but they are better ideas because of it.

3. Love what you do. Let’s face it, you will earn less (a lot less) than your friends who went to law school (or than Trip, who is founder and CEO of Scribd, one of the hottest start-ups of 2009). You will not become famous (Trip has his own Wikipedia page and gets interviewed on NBC, I do not). You are doing science for the fun of it. I deeply cherish (and now mostly reminisce about) those moments of burning intensity when faced with a novel discovery. The times when you look under the microscope and utter, “Oh my god…”

4. Give chance a chance. As trite as this may sound, one has to be prepared for the unexpected. Carefully laid out plans may work out well for some (they certainly help with getting fundable scores on grants), but random events big and small can open up horizons one never envisioned.

ACKNOWLEDGMENTS

I am forever grateful to my mentors Karsten Weis, Rebecca Heald, and Peter Jackson for giving me the freedom to pursue what might have seem like random directions at the time. Sitting now at the other side of the desk, I can fully appreciate the challenges of providing scientific freedom to lab members, while keeping a coherent research program supported by renewable grants. Thank you all; I could never have made it to where I am now without your generosity and support. Research in the Nachury lab is supported by grants from National Institutes of Health/National Institute of General Medical Science, the March of Dimes, the American Heart Association, a Searle scholar award, a Sloan fellowship, and a Klingenstein fellowship.

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Science stories: flies, planes, worms, and lasers

Melissa Rolls
Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

ABSTRACT “Tell a story,” my mother instructs her graduate students as they prepare their talks. I will make use of her advice here, and will tell several short stories. The themes revolve around the practice of science—what motivates us to go into science and how we choose questions once we get there. I also touch on progress in scientific tools, teaching, good mentors, and good colleagues, all of which contribute to making a career in science constantly compelling.

FLIES ON PLANES
An enthusiastic undergraduate at the University of Pennsylvania decides she wants to try a genetics project. She signs up for independent study with a fly lab. Pretty soon, she and another student are ready to travel to Brookhaven to irradiate the flies. The students and flies are picked up in Philadelphia by a limo, taken to a small airport, and loaded onto a private plane. They get a great view of the coast as they travel north. At the other end, they are once again met by a limo. What a way to mutate some flies! The undergrad decides science is pretty good and embarks on it as a career. This undergrad was my mother. She is still a scientist, at Penn State, like me. Although she did not continue to work on flies, I do. But it is no longer standard practice to transport your flies on private planes or coddle them with limo rides. We have a different set of luxuries now.

THE LUXURY OF NEW EXPERIMENTAL TOOLS
The day-to-day practice of being a scientist has definitely changed in the past 40 years. Aside from losing luxury travel and gaining computers, we have a lot more tools for our experiments. Because students often take these tools for granted, it is particularly satisfying to teach about older alternatives and the transformative capacity of the new methods.

One of my favorite questions to ask my undergrad class when I show them a picture of fluorescently labeled cells from 1970 is, “How was this figure made?” The students are stumped. We look at one another in silence for a while. Then I remind them that there were no computers involved. No charge-coupled device (CCD) cameras were on the microscope. Maybe someone mentions film. If they get this, I’ll help them out: “The microscope had a film camera mounted on it. The person took a picture, then took the film to a darkroom and developed it to see if the image looked good. Then what?” More silence. They almost never get the next part: scissors, glue onto paper, and rephotograph to make the final figure, then send in three prints of the figure for the reviewers. This exchange always makes me gleeful. Maybe it is because I really hated film and love being able to see my cells on the computer immediately, thanks to CCD cameras and imaging software.

Some other science tools that are particularly satisfying to teach are green fluorescent proteins (GFP), RNA interference, genome sequences, and PCR. The tools are very tangible evidence of the huge progress that has been made in the biological sciences.

MATCHING TOOLS WITH QUESTIONS
Of course, having good tools is only useful if you have something compelling to do with them. During my research training, I was lucky enough to have four great mentors: Carolyn Machamer, Jack Rose, Tom Rapoport, and Chris Doe, who mixed guidance and freedom as needed. This means that at various times I had to think hard about what I really wanted to tackle.
When I decided to join Tom’s lab, he told me that I could pick anything I wanted to work on, with one condition: I had to aim to reconstitute it in vitro from purified components before I was done. I failed in all parts of this challenge. I floundered thinking of something to work on, and ended up joining forces with another graduate student, Pascal Stein, on a project. We started off in the test tube, trying to reconstitute nuclear envelope assembly with frog egg extracts. It did not go well. After a year, Pascal suggested we try to identify new nuclear envelope proteins using a strategy that Stephen Taylor had developed in Frank McKeon’s lab. We moved out of the test tube and into whole cells to screen a GFP-cDNA library. This worked out better, and we found a new protein (Rolls et al., 1999). Pascal followed up on this project, and I was left once more to wonder: What question did I really want to ask? This time I came up with one. In addition to working together on a project, Pascal and I had started a journal club in the lab. As a result of reading and discussing papers in this group, I had developed an idea about where to go next with my project.

I wanted to know how rough endoplasmic reticulum (RER) proteins are kept out of the smooth ER (SER), whether the ER is continuous. I tried pilot studies in mammalian cultured cells, but they did not look promising. What I needed was a cell type in which the RER and SER were spatially separate, so I could distinguish them by light microscopy. Muscle cells or neurons seemed most likely to meet this criterion, as they have large regions of SER. I considered two options for looking at these tissues: cultures of differentiated mammalian cells or whole Caenorhabditis elegans. After some pilot studies, I decided C. elegans would be best, as I could look at both muscle cells and neurons using the same methods. The cells would be fully differentiated and functional in a see-through body, without any finicky culturing. I generated a new set of markers for C. elegans membranes, including RER, SER, Golgi, and nuclear envelope. The system was great! The only snags were that I did not figure out how domain formation in the ER worked, only one way it did not work (Rolls et al., 2002), and also there was no way this project was going to end up with an in vitro reconstitution. Two top lessons from this experience were: 1) choose the best system/tools to answer the question, and 2) cells can do very sophisticated things in vivo that they do not always do well in culture.

CHOOSING A NEW AREA IN A NEW LAB

Stay focused.

If you are a new principal investigator starting your own lab, I know you have heard this. I hope that being somewhat focused but totally open to ideas from great colleagues and students works as well!

When I started my lab at Penn State, there was one question that was begging to be answered first. As a postdoc, I had found that microtubules in Drosophila sensory dendrites have microtubules with minus end–out polarity (Rolls et al., 2007), not the mixed polarity found in cultured mammalian neurons (Baas et al., 1988). I wanted to know whether this was limited to sensory neurons, or whether all Drosophila neurons would share this microtubule organization. While Michelle Stone tackled this question (Stone et al., 2008), we read a variety of papers about microtubules in neurons. One paper we really enjoyed showed that microtubules are dramatically rearranged near the cut site after injury of an Aplysia axon in culture (Erez et al., 2007). Michelle asked me whether we could use our Drosophila tools to see whether something like that might happen to an injured neuron in vivo. She explained that when she was growing up her dad had a stroke, and it had changed him and her family forever. She wanted to do something to understand what went on in his brain during and after the stroke. I thought about it and answered, “Get your first paper published, then play. We’ll see if we can come up with a way to injure the neurons in vivo.”

Mark Terasaki, whom I met when I was in grad school, had described to me a method to cut axons in vivo using a two-photon laser (Galbraith and Terasaki, 2003). It turns out those lasers are expensive and break a lot. But at some point I remembered that most C. elegans labs have lasers to ablate cells, and if you can kill a cell, you should be able to cut an axon (C. elegans labs had also realized this [Wu et al., 2007]). My next-door neighbor, Wendy Hanna-Rose, is a worm person and a fabulous colleague and had a pulsed ultraviolet laser. The laser worked to cut axons, and she let us put it on our scope. Almost immediately, Michelle noticed not just local changes near the axon cut site, but an increase in the number of growing microtubules throughout the entire dendritic tree (Stone et al., 2010). This change was so striking that she ran into my office the first time she saw it and told me I had to come see right away. Although we are now less focused on neuronal polarity, we are completely hooked on understanding neuronal responses to injury—something that would not have happened without several brains working together.

A LAST SHORT STORY ON BEING INTRODUCED TO THE ASCB

I started in Carolyn Machamer’s lab at Johns Hopkins the summer before my freshman year at Yale. In my second summer, she announced at a lab meeting, “Whoever wants to go to the ASCB meeting this year needs to give me an abstract to look at by next week.” I asked her whether this included me, since I was just a summer student. She froze and looked surprised as she thought about this, but replied after a while that the offer was open to anyone who could come up with a reasonable abstract. So I went, and I have gone back almost every year since then to learn about new tools and ideas and to hear great stories. I am still grateful to Carolyn for taking the chance on an inexperienced undergrad. Following her example, we typically have 8–10 undergrads in the lab and constantly benefit from their enthusiasm and openness to new ideas. I have not yet brought any of them to the ASCB meeting, but none has asked yet. A few last morals: if you do not ask, the answer is no; be open to new people and ideas; and, of course, enjoy your science!

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Spatial and temporal impacts on a career in science

Susan R. Wente
Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

ABSTRACT In cell biology, subcellular locale is critical for the action of signaling molecules, for regulation of gene expression, and for proper cell division. In simple terms, everything must be in the right place at the right time. For my research, I have focused on understanding the role the nuclear pore complex (NPC) plays in maintaining this balance. With eukaryotic transcription in the nucleus and translation in the cytoplasm, highly selective import and export events at the NPC connect these spatially separated processes to allow gene expression. In a similar way, spatial and temporal events have repeatedly impacted my scientific career. In different places and times, interactions with mentors, collaborators, colleagues, and trainees have shaped my research and mentoring philosophies: aim high, fuel your passions, collaborate, and take risks to find supportive environments and challenging projects that impact scientific discovery.

INTRODUCTION

D. W. Fawcett’s (1981) book The Cell, with its elegant electron micrographs and drawings of cellular structures, has been a mainstay of my bookshelf for the past 20 years. It is always available to show new students the striking subcellular architectures that are so critical to spatial and temporal regulation of cellular physiology. Like the organelles, proteins, and RNAs in a cell, I have been most fortunate to find myself in the right place at the right time. In receiving the 2011 Women in Cell Biology Senior Award, I wish to share this honor with all I have worked with in different places and all who have inspired me at many different times.

A PASSION FOR PROTEINS FROM THE HEARTLAND

Raised in a small Iowa town, I was instilled by my parents with a love of education and a strong work ethic. I arrived at the University of Iowa in 1980 as an undergraduate student with a simple low-risk career plan—register as a predental hygiene major and finish with a stable job. Clearly, I’ve learned to aim my goals higher, due to mentoring by many. The late Gene Lata, the undergraduate advisor in the Department of Biochemistry, guided me to a work-study job in F. Jeffrey Field’s laboratory studying rabbit cholesterol esterase activity, and then to an honors thesis with Alice Fulton, the first woman faculty member in the department. Fulton’s passion for research was contagious, and I was completely hooked on studying (cytoskeletal) proteins. Encouraged to explore, I did a summer internship at Brookhaven National Laboratory working with Marshall Elzinga on structural properties of actin. With growing confidence and a focus on protein biochemistry, I left Iowa for graduate school at the University of California at Berkeley.

Moving to Berkeley was a big leap out of the heartland. Fellow students at the time made this a truly exciting place, and here I met my future husband, Chris Hardy. In research, site-directed mutagenesis technology offered a new way to study protein function. I had the privilege of working with Howard Schachman, a statesman of protein biophysics, and we merged this brand-new technology with classic approaches (e.g., analytical ultracentrifugation with the Model E) to dissect catalytic and regulatory properties of aspartate transcarbamoylase (ATCase). Schachman trusted his students to be independent while inspiring creativity and active debate on
allostERICs. He coupled this with regular instruction on the ethics of research and the navigation of politics in science. This environment solidified my passion for research and provided a vision for leading and mentoring.

**FUELED BY THE NUCLEAR PORE**

When I began considering postdoctoral training, the Berkeley faculty challenged me to bring my studies of protein mechanism into the context of cell function. So, with my new husband and I focused on New York City as the next place for our careers, I decided to work at Memorial Sloan Kettering Cancer Center with the late Ora Rosen, an inspiring model as scientist and mother. With the gene encoding the human insulin receptor just cloned, I planned to merge my enzymology expertise with studies of its signaling networks. This was cut short by Rosen's untimely death, bringing doubts: should I consider an alternative career? I did interview at a patent law firm, but an opportune meeting with Günter Blobel at Rockefeller University restored my passion and confidence. I became fascinated by Blobel's landmark work on the machinery for trafficking across organelle membranes, especially efforts on the nuclear envelope. If bacterial ATCase (assembled from a dimer of trimers and a trimer of dimers) had provided decades of challenges to scientists, I reasoned that the nuclear pore complex (NPC) puzzle—then speculated to involve ~100 different polypeptides in unknown octameric subassemblies—could be the fuel for refocusing my career. Blobel and his laboratory welcomed me, and I bought Fawcett's The Cell to review cell biology and anchor my career.

In 1990, tools to study NPC structure and function were few. A fellow postdoc, Mike Rout, soon persuaded me to merge our strengths in discovering yeast NPC proteins (Nups). This was my first serious scientific collaboration. Mike pioneered the purification of NPCs, and I focused on expression-based cloning strategies. With candidate genes in hand, we could rapidly confirm the individual protein's localization at NPCs and reveal function with yeast genetics and electron microscopy. On the basis of comparison to the few then-known Nups, we identified conserved N-terminal domains with tetrapeptide GLFG repeats distinct from FxFG or FG motifs in others. Suddenly, a picture of the NPC as composed of protein families emerged, and we forged ahead to uncover more players.

**NETWORKING FORWARD FROM THE 1991 AMERICAN SOCIETY FOR CELL BIOLOGY MEETING**

My introduction to the American Society for Cell Biology proved fateful: while I was visiting the poster presentation of an Iowa mentor, Peter Rubenstein, at the 1991 meeting, he introduced me to John Cooper with the message, “If you want to hire someone….” Cooper came to my poster presentation the next day, and I soon received an invitation from Phil Stahl to apply for a faculty position in the Department of Cell Biology and Physiology at Washington University in St. Louis. It was a serendipitous lesson in networking. I arrived there in the fall of 1993, the second woman hired, with the first (Maurine Linder) a touchstone for building my lab and mentoring students. The department provided a stimulating and supportive environment—the right time and place for beginning an academic career and starting a family.

My laboratory was launched with the goal of studying the highly selective, bidirectional exchange of proteins and RNA through the NPC, including both NPC transport and biogenesis mechanisms. My first students, Kathy Iovine and Rob Murphy, started projects based solely on the GLFG Nup family in the yeast Saccharomyces cerevisiae. For this, the GLFG Nups were in the right place—serving as docking sites for nuclear transport factors. Indeed, they are now central to the models for NPC translocation and have been the seeds for dozens of Ph.D. and fellow projects.

To make discoveries, we took risks and exploited the newest technologies coupled with classic approaches (cell biology, genetics, biochemistry)—just as I learned in graduate school. As an example, when green fluorescent protein (GFP) was first reported, Mirella Bucci used a GFP-tagged GLFG Nup to do assays of live-cell NPC dynamics. This set the stage for the first forward genetic screens, in which Bucci and then Kathy Ryan identified mutants with mislocalized GFP-Nups and assembly defects. The mutants yielded critical in vivo evidence for the involvement of Ran and karyopherins in NPC biogenesis. We were also encouraged by the success in Hardy's lab with early synthetic lethal genetic screening technology. Murphy applied the approach to a GLFG nup mutant and identified a novel mRNA export factor, Gle1. His second-generation synthetic lethal screen with a gle1 mutant then led to an unexpected connection to phospholipase Plc1. Sharing our unpublished data on the gle1 plc1 mutant with one of my Berkeley professors, Jeremy Thorner, led him to reconnect me with John York at Duke University.

Ironically, York and I first met when he trained at Washington University (and he too is an Iowa Biochemistry Department alumnus). Thus, we already had spatial and temporal connections. With York's expertise on inositol signaling and our other gle1 synthetic lethal mutants, we discovered the genes encoding long-sought-after kinases for inositol hexakisphosphate (IP₆) production. Moreover, this result immediately suggested a physiological function for IP₆ mRNA export. It was exciting to see these breakthroughs from combining our laboratories' strengths. More so, this again connected several recurring themes in my research and career: the essential roles of networking and collaborating.

**MOVES TO NEW MODELS AND ROLES**

In the summer of 2002, I moved to Vanderbilt University School of Medicine to chair the Department of Cell and Developmental Biology. This opportunity found me by surprise, but I became intrigued with building a progressive and collaborative environment by recruiting and mentoring faculty. This felt like a natural extension of working with students and directing graduate programs. I recognized that Vanderbilt was also the right place at the right time—there were multiple women in leadership roles, and the institution was committed to growing in the basic sciences and launching new transdisciplinary initiatives.

In this new place, my trainees again embraced my message to tackle new approaches and model systems, from yeasts to human cell culture to zebrafish. Over the years, the FG Nup family expanded to 11 members; thus, to analyze in vivo function exhaustively, we had to develop an innovative strategy. With a huge investment of effort by Lisa Strawn and Laura Terry, >300 S. cerevisiae mutants with double, triple, and multiple higher-order in-frame deletions of FG domains were made and tested for growth and transport defects. Several fundamental “rules” emerged: only a subset of the FG domains are essential, and different transport factors preferentially use different FG “pathways” through the pore. At the same time, we became keenly interested in defining the molecular sequence of events for mRNA export at the NPC. Building on our work with both yeast and human Gle1, Abel Alcazar-Roman and Beth Tran used a combination of genetic and in vitro reconstitution assays to show that Gle1 bound to IP₆ activates the Dbp5 ATPase for remodeling mRNA–protein complexes at the cytoplasmic NPC face. We and others have speculated that this spatial control of Dbp5 is important for directional export. However, we also knew that Gle1 and IP₆ were dynamic, and Tim Bolger soon found that Gle1 and IP₆...
act not just at the NPC, but also in the cytoplasm during translation. We were excited that this significantly extended the paradigm for coupling multiple steps in the gene expression pathway, with factors acting in more than one cellular place and process.

TOWARD COMING FULL CIRCLE
When my hometown newspaper reported that I was at Vanderbilt, my parents received a call from a farmer in a town 10 miles south. Coincidentally, his son, Bruce Appel, was then on the faculty—we had grown up at the same time in virtually the same place. Appel welcomed me to Vanderbilt by sharing his favorite model—the zebrafish, Danio rerio—and adopting my postdoc, Bhaskarjyoti Sarmah. Using zebrafish, we found novel roles for the IP_6 pathway in ciliary function and hedgehog signaling during early development. This work not only opened up entirely new areas of research, it also positioned us for the unexpected. A couple of years ago, a Finnish group reported an unexpected genetic linkage of a lethal human fetal disease to Gle1 (Nousianinen et al., 2008). With our 12 years of basic discovery research on Gle1, mechanisms for the pathophysiology could immediately be proposed, and we are now leveraging our zebrafish experience to develop a disease model. It has been gratifying to see our work start to come full circle—from discovering a novel factor in yeast to understanding human disease.

All of these past times and places have guided my approach to research and mentoring. The career lessons I pass on always include encouraging others to aim high and to build their confidence to take risks. Finding stimulating constructive environments with supportive colleagues is key to this process, as is collaborating and sharing research. For me, it has truly been a great privilege to have an opportunity to help others’ careers and to make discoveries. As for balancing research, administration, and personal roles, to me, each is an essential “organelle” of my life and career. Thus, each day is an opportunity to prioritize, organize, and make choices about what is most important in the given time and place.

ACKNOWLEDGMENTS
I am indebted to my generous mentors, collaborators, and colleagues for their sharing of time, place, and lessons; to all the students, fellows, and staff who have worked with me and who dedicated their efforts (both named and unnamed in this essay); and to my supportive family, especially my husband—a partner who has made sacrifices for my career all along the way—and my daughters—good-luck charms who have given me perspective. I am thankful for the tremendous support from multiple funding agencies—the Arnold and Mabel Beckman Foundation, the American Cancer Society, and the Steven and Michele Kirsch Foundation for awards at early and midcareer points; the American Heart Association and March of Dimes for supporting our recent zebrafish work; and, of special importance, the National Institutes of Health for continuous funding of my program and my trainees.

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A different kind of quarterback

Jerry Charles Guyden
City College of New York, New York, NY 10031

ABSTRACT I am not big on celebrations, nor do I accept many invitations to receive awards. There is much work to be done, and the reward is in the doing. I learned this lesson early from my parents, Martha and Robert Guyden. However, I am humbled that anyone would even mention my name in association with E. E. Just. I, like he, was born into a segregated America, and somehow we both found biology. I think Just’s life story instigates a discussion on diversity in science, as well it should. However, after reading Tyrone Hayes’ (2010 E. E. Just Award recipient) essay from last year, “Diversifying the Biological Sciences: Past Efforts and Future Challenges” (Hayes, 2010), I have little to add on the subject. His words gave voice to my thoughts. That being said, I would like to use these pages to describe my journey into the “Cell” and the people who “hoed the row ahead of me.”

I integrated myself into college. There were a few majority schools in Texas that accepted African Americans in 1970. It was not until 1972 that public integration was forced in Texas. My dream then was of becoming the first black NFL quarterback. Although I finished second in my class in high school academically, football was/is a religion in Texas, and playing ‘ball had always been the primary focus of my life. I soon learned that America was not yet ready for a black quarterback at a majority college, and becoming an NFL quarterback was literally impossible. I realized that this was the end of my childhood dream, but could not give up athletics altogether. So I joined the track team, and for the next 2 years I majored in pole-vaulting, with a minor in math. Late in my sophomore year, I became conscious of the fact that there were no professional pole-vaulters in the United States, and math was not my cup of tea. Although my mother and brother were mathematicians, in my mind there were no straight lines in linear equations.

Now in my sophomore year of college, I had to figure out who I wanted to be, and I had no images of me in the future. I remember becoming ill while visiting my parents. I saw our family doctor the next day. He suggested an alternative, a career in medicine. And that was that. I would become a physician. I returned to school with a new purpose. My grades improved significantly. I was the only African American premed major at North Texas State University at that time, and I made sure I competed at the top of the class as I had plans of applying to medical school.

I worked hard to develop relationships with my classmates. We created study groups, many of which I led. Our test scores were always at the top of each class. In those days, comparative anatomy and physiology were the most difficult premed requirements. These classes were taught by one professor who also chaired the premed committee. At North Texas, candidates for medical school were required to apply through the campus premed committee. The unwritten rule was that your work had to meet the standards of the chair, and his primary requirement was that you aced his classes. The anatomy class consisted of both a lecture and a lab. I led most of our study sessions, whether the subject matter was associated with lab topics or lectures. Although I had one of the highest grades in lab, I received grades that were lower than other members of our study group on lecture exams. We
compared grades after each exam, and it was clear that I received fewer points for the same answers given by other members of the group. In fact, some of my tutees received higher scores than I. At the end of the semester, I received a “B” for the class. This professor was very strict with respect to grades. Ninety was an “A” and 89 was a “B.” My grade average was 89.7. Every one else in the study group received grade averages above 92.

Again, now in the spring of my junior year, images of me in the future were taken away. Using anger as the prime motivator for my efforts, I continued to do well academically. In the last semester of my senior year, I was required to take genetics to complete the pre-med core. I segregated myself from the rest of the class by sitting in the far back of the class. Several rows separated me from my classmates. I promised myself that I would perform better than anyone in the class on every exam. Sometime in the middle of the semester, Professor David Busbee took longer than usual grading an exam. One of the “smartest” students in the class asked why he had not returned the graded exams. Dr. Busbee replied that the exams were graded, but not yet recorded in his grade book. The student then asked who made the highest score? Dr. Busbee answered in a very loud and booming voice, “Jerry Guyden.” Now, this got my attention, and, as he continued to look at me, he asked if he could talk to me after class.

To this day, I do not know why Dr. Busbee asked me to work with him in his lab. I knew very little about research, and I knew nothing of his research interests. I knew I had to wear a white lab jacket, and I thought it would make me look good, kind of like a physician. More importantly, he said he would pay me, so I said yes. I soon learned that Dr. Busbee studied cancer. He gave me a large stack of papers and told me to read them before I could start my own research project. I was also required to attend lab meetings. One of the strangest concepts for me was getting paid to study. I checked every day with Dr. Busbee to assure myself that I was doing the right things to get paid. I thought work in labs involved something with test tubes and microscopes, like in lab classes. I knew no one who was a scientist. They did not exist in my world. Initially, this was nothing more than a summer job.

The Dr. Busbee lab studied the activity of aryl hydrocarbon hydroxylases. Many flat and planar multi-benzene ringed chemicals found in tar and cigarette smoke are metabolized by aryl hydrocarbon hydroxylases in the body to convert these potentially harmful chemicals into removable waste. Some of these metabolites, however, interact strongly with DNA. Now, for a long time, I had no clue about what was going on, but I did not want to be embarrassed in lab meetings, so I read extensively. In retrospect, I think most beginning scientists feel this way. One day, I thought I would give it try. I thought I knew enough to design an experiment to isolate one of the metabolites of interest. I do not remember the details of the experiment, but I do remember the results. In the old days (before computers), the high-performance liquid chromatograph was attached to a ticker-tape machine. The results of an experiment were displayed on a long strip of paper streaming from the machine. I found myself, early one Saturday morning, standing in a small, dimly lit room awaiting my results as they came off the ticker-tape machine. I remember saying to myself, “It worked! It worked!” My next thought was, “Why am I here working so early on a Saturday morning?” The answer to this question changed my life forever. I reasoned that I was there because I wanted to be, that I was excited about my experiment...its results. I realized that I initiated the idea, created a plan, and executed the plan successfully. I was a quarterback again, and no one could take my hard work, thoughts, and ideas away from me. Visions of me in the future crystallized that Saturday morning.

The results of my work with Dr. Busbee produced two papers and a master’s degree. During the last year of my master’s program, I received a phone call (out of the blue) from a stranger. His name was Lovell Jones (you may remember him from last year’s E. E. Just essay). He asked if I was interested in applying to the doctoral program in zoology at the University of California, Berkeley. I thought this was some kind of prank. How could anyone at Berkeley know me, my work? Neither was that important. He offered me a visit to the campus. He scheduled meetings with several faculty members, and he promised that if I joined the PhD program at Berkeley, I would have a fair chance of successfully completing the program. A fair chance was all I wanted.

During that visit, I met Steve Martin. Steve is British, and I was this young African American from Texas. Our initial conversation consisted mostly of “huh.” It was like he was Russian and I was Caribbean speaking patois. Somehow we got through the conversation. I immediately realized that he was not only one of the brightest men I had ever met, but also he was an honest and good human being. He asked me to join his lab and I did. I do not think that Steve ever asked me to do anything throughout my entire tenure in the Martin lab. Steve taught by example. He did his work, and if you wanted to be a part of the lab, you had to keep up. That meant you had to read, design experiments, write proposals, and perform the experiments. I remember my first proposal looked like a stop sign when he returned it. I thought my writing abilities were at least adequate and took offense at several of his comments. I hurried back into his office and soon discovered that I had a long way to go to compete at his skill level. But that day, he threw down the gauntlet and presented the challenge. I like challenges. The work in the Martin lab was centered on oncogenes. We discovered that several retroviruses carry genes that produce cancer-causing proteins. Our work produced two papers, one in Cell, the other in Virology, and a PhD.

During my last year with Steve, I began looking for a postdoc position. Leon Wofsy in the Department of Microbiology and Immunology at Berkeley had a postdoc position open. I asked for an interview, and after our discussions, I realized that he was not very impressed when he said he would get back to me. What was this, and who was he? I thought I was the best thing since the discovery of black pepper. How could he not want me to work with him? I called and asked for another interview. This time our discussions were extensive, and they existed on two levels. First, we talked about the science, and he decided that I was capable and hired me for the job. We also talked about life issues. I discovered Leon was Jewish and from New York City. He came to Berkeley after finishing his undergraduate degree from the City College of New York. I also learned that he had marched in the civil rights movement led by Martin Luther King. One of his friends was Paul Robeson. He had autographed drawings from the famous artist Charles White, and he played ball in Grove Park with members of the Black Panthers. He also testified in front of Congress and J. Edgar Hoover’s FBI. I knew I could learn from this guy, and not just science. In that moment, I also realized that he saw me as a human being, not a black man, but a man. Leon was looking for the best person for the job. If you happened to be black, cool. But being black was not one of the qualifications for the job. Being a good scientist was. He knew enough about black people, all people for that matter, to express his true feelings about my qualifications without fear of inadvertent cultural insults. So he treated me the way he treated every candidate. It was up to me to prove my worth as a scientist. During my time with Leon, we developed the first method for antibody-toxin targeting and killing of cells. It was Leon’s idea to create a “silver bullet” specifically directed at cancer.
cells. I must say that the science was amazing, but paled in comparison to his life lessons. It was Leon who influenced me to take a faculty position at the City College of New York.

During my 25 years here at the City College of New York, I have attempted to achieve two goals. First, I wanted to study the function of thymic nurse cells (TNCs), and second, to provide a safe and judgment-free zone to introduce young minds to science. TNCs are stromal epithelial cells of the thymic cortex. They are very unusual, because they internalize up to 200 developing T cells into specialized “cytoplasmic” vacuoles. Of equal importance is my commitment to training students for careers in science. We have successfully trained 68 young men and women from several different ethnic backgrounds and races (Caucasian, African American, Hispanic, and Asian) in my laboratory. Forty-eight were minority students. Individuals were trained at all levels of the educational process, from undergraduate to postdoctoral fellow.

Twenty-four of the students from underrepresented communities now have either a PhD or an MD or both degrees. Twenty-four are still in the pipeline. Our training methods have been successful and require the development of teams of people representing several different cultures and ethnic backgrounds working together toward accomplishing a scientific goal. Individuals working together day-to-day find common ground and establish lifelong relationships that will influence the cultural makeup of the next generation of scientists. I will continue to address the issue of preparing the next generation of scientists in the United States, which should include a significant increase in the number of individuals from all ethnic and racial backgrounds.

REFERENCE
As cell biologists, like other scientists, we rely on paradigms for guiding our inquiries into how cells function. By paradigm, I don’t mean a specific model, which may be expected in many instances to be overturned altogether, but a deeper conceptual view, interconnected with the technology and scientific language of the times and unquestioned by the majority in the field. For example, the view that all eukaryotic cells have a secretory pathway comprised of a stable endoplasmic reticulum (ER) and Golgi apparatus, using discrete transport vesicles in order to exchange their contents, was long believed to be fundamental for further research. The history of cell biology is marked by acceptance of such paradigms, but also by their gradual evolution.

Having a penchant for philosophy since undergraduate days, I’ve often wondered how paradigms in science come about and what prompts their modification. In my experience, the answer has a lot to do with 1) available technology, which circumscribes what is tested and hypothesized in science, and 2) shared scientific language in a particular field, which influences how findings are related, how scientific phenomena are conceived, and how research questions are framed.

The secretory membrane pathway in my view may be such an example of an evolving paradigm in cell biology. Classic experiments using electron microscopy (Dalton and Felix, 1954; Farquhar et al., 1974; Rambourg et al., 1979) and pulse-chase autoradiographic tracing of newly synthesized proteins (Neutra and Leblond, 1966) set the framework of this paradigm, revealing the progressive movement of newly synthesized secretory proteins from the ER to the Golgi to the cell surface. Small vesicles in the vicinity of the ER and Golgi apparatus seen in transmission electron micrograph cross sections were interpreted as transport intermediates, conveying proteins from one stable station to the next before reaching the plasma membrane. With this framework in hand (Farquhar and Palade, 1981), researchers in the 1980s began addressing the complexity of the intracellular membrane transactions involved in secretory transport, following three distinct research strategies—biochemical, genetic, and imaging approaches.

Pioneering the biochemical approach was James Rothman and colleagues, who used cell-free extracts to reconstitute vesicle formation and fusion of Golgi-derived membranes (Balch et al., 1984). Examining oligosaccharide processing in mixed extracts from mutant “donor” and wild-type “acceptor” Golgi membranes, they found that the virally encoded vesicular stomatitis virus glycoprotein (VSVG) underwent sequential carbohydrate processing, prompting the conclusion that the glycosylation machinery is compartmentalized across
the Golgi’s stack of cisternal elements. In the framework of a stable Golgi system, this led to the idea that vesicles carry VSVG across the Golgi stack in a cis-to-trans direction. Using the in vitro transport assay, Rothman’s group then purified cytosolic components necessary for vesicle budding and membrane fusion (Serafini et al., 1991; Waters et al., 1991; Sollner et al., 1993). Concurrently, Schekman and colleagues spearheaded the genetic approach, isolating conditional lethal, temperature-sensitive secretion mutants and mapping the localization of the corresponding gene products in yeast cells (Novick et al., 1980, 1981). With this, Schekman’s group produced a temporal map of the secretory pathway, identifying the genes and proteins required to operate the secretory pathway (Kaiser and Schekman, 1990). The two approaches soon converged in identifying a core molecular machinery involved in controlling how vesicles form, translocate, and fuse among donor and acceptor compartments of the secretory pathway. The result was a beautiful synergy, reinforcing the prevailing view that protein secretion involves the activity of small transport vesicles for intercompartmental trafficking of proteins. Expressions such as anterograde transport, COPI- and COPII-coated vesicles, small GTPases, and soluble N-ethylmaleimide–sensitive factor attachment protein receptors (SNAREs) became the indispensable vocabulary in the field.

The third approach—imaging—initially served a supportive role to the dramatic advances made by the biochemical and genetic approaches. Static imaging by electron microscopy (EM) of secretory membranes gave strong support to the idea that COPI vesicles shuttle proteins between stable Golgi cisternae (Orci et al., 1989). However, as the capability to image dynamic processes developed, new ideas emerged. Researchers employing imaging in the late 1980s at first were less interested in the mechanistic basis of secretory vesicle traffic than in the nature of the organelles and vesicle intermediates themselves. Entranced by the elegant morphology of the Golgi’s elaborate, stack-like structure and the web-like network of the ER, now viewable because of new immunofluorescence techniques, such researchers wanted to address whether organelles are stable, independent structures or more dynamic, relying on other compartments. One hint that the Golgi might not be stable came with the immuno-EM findings by Graham Warren and John Lucocq in the late 1980s showing that the Golgi partially disassembles during mitosis and then reforms (Lucocq and Warren, 1987). However, from the perspective of the governing paradigm, the EM images of scattered mitotic Golgi elements were interpreted as evidence that the Golgi is capable of vesiculating during mitosis and then reforming through the reassociation of these fragments.

Observations with the fungal metabolite brefeldin A (BFA) were more difficult to accommodate. In Rick Klausner’s lab, where I worked as a postdoc, we observed that the Golgi tubulates upon addition of BFA (Lippincott-Schwartz et al., 1990). The tubules carried Golgi proteins back into the ER and no apparent Golgi persisted after minutes of BFA treatment. This was the reverse of the forward-only paradigm of vesicle trafficking; moreover, the transport back to the ER did not require conventional coat protein machinery (Donaldson et al., 1990; Orci et al., 1991). When BFA was removed from cells, there emerged a new, fully functional Golgi. These findings did not neatly fit into the biochemical/genetic-based advances in the secretory transport paradigm, in which coated vesicles and small GTPases were seen as central to all trafficking within a stable Golgi system. Instead, the results suggested there are mechanistically distinct anterograde and retrograde trafficking pathways operating between the ER and Golgi (Klausner et al., 1992). These pathways use both vesicular and non-vesicular transport carriers, and a balance in membrane flux between the pathways determined Golgi size and existence.

Some dismissed the BFA results as an artifact of the intervention into cellular processes with the drug. With the advent of the green fluorescent protein (GFP) revolution in which proteins could be tagged in living cells and visualized as they moved through the secretory pathway, it became possible to image the secretory pathway in vivo without BFA. This yielded several surprises in light of the prevailing paradigm. When cargo transport through the secretory pathway was visualized, instead of small vesicles randomly diffusing to and from the different organelles, large tubular-vesicular structures were seen conveying cargo from the ER to Golgi apparatus (Presley et al., 1997). The structures used molecular motors to move along microtubules toward the Golgi and varied in size depending on levels of cargo flux through the pathway. When I first presented movies showing the trafficking of VSVG protein at a conference in 1997, Ben Lewin, then Editor-in-Chief at Cell, memorably asked, “Where are the vesicles?,“ to which one member of the audience replied, “They must be invisible with GFP.” The movies revitalized the idea of cisternal progression from EM studies of the 1960s (Morre and Mollenhauer, 2007), since the pleomorphic transport intermediates appeared to fuse together upon reaching the Golgi apparatus.

However, the GFP-based movies also raised deeper questions. Quantitative measurements of VSVG-GFP trafficking through the secretory pathway by Koty Hirschberg in my lab revealed no change in the rate law for VSVG export out of the ER or Golgi as the number of VSVG molecules in these compartments dropped from >20 million to tens of molecules after temperature release from the ER (Hirschberg et al., 1998). This suggested that the rate-limiting steps in transport do not depend on binary interactions between cargo and specific transport components, which should show saturation effects. One possibility is that they depend instead on lipid phase separation processes, which do not respond to dilution. This fit with emerging membrane lipid research suggesting that the lipid bilayer is not a structurally passive solvent but exhibits lateral segregation potential due to the preferential association among sphingolipids, sterols, and specific proteins (Simons and Ikonnen, 1997). Self-organization of lipids and proteins in the bilayer in this model is believed to induce subcompartmentalization to organize bioactivity of cell membranes. This could drive membrane trafficking events in a way that explains the single rate laws observed for VSVG-GFP trafficking kinetics

Using GFP-based photobleaching and kinetic modeling approaches, researchers in my lab and Cathy Jackson studied the membrane binding/release kinetics of different coat protein components responsible for cargo sorting into carriers (including COPI, Arf1, ArfGAP1, and GBF1). All of these proteins underwent fast cytosol/membrane exchange irrespective of vesicle budding (Presley et al., 2002; Liu et al., 2005; Niu et al., 2005). This suggested that the formation of a “coated” carrier occurs on a different time scale than the binding/release cycle of individual coat components, implying that coat lattices are metastable and may not immediately disassemble. This property is similar to that in other filamentous systems, such as microtubules and actin. The effects of metastable coat lattices on membranes are unclear, but one possibility is that they affect protein retention in the Golgi by exerting membrane tension in the bilayer (Antonny, 2006). Biophysical studies of model membranes have shown that membrane tension can drive large-scale phase separation and sorting of lipids and proteins (Baumgart et al., 2003; Roux et al., 2005; Manneville et al., 2008). Without such tension, sorting/segregation of molecules is disrupted. One way to explain the nonselective, directed flow of Golgi membrane components back to the ER under BFA treatment, when coat proteins are dislodged from membranes, is by this type of mechanism.
Using fluorescence recovery after photobleaching to measure the residency time and trafficking pathways of different GFP-tagged Golgi resident components, Theresa Ward in my lab showed they all were transiently associated with Golgi membranes and underwent either cycling through the ER (i.e., transmembrane enzymes and itinerant membrane components) or rapid exchange with cytosolic pools (i.e., Golgi coat and matrix proteins; Ward et al., 2001). We further demonstrated that within Golgi membranes Golgi enzymes undergo rapid lateral diffusion and are unhindered by extensive interactions that “fix” these proteins within different cisternae (Cole et al., 1996). In addition, Golgi enzymes were shown to move between Golgi elements by membrane tubules (Sicaky et al., 1997), which extended between Golgi subcompartments (Trucco et al., 2004). These findings raised the question of how the Golgi maintains itself as an organelle, retaining its resident components to prevent them from flowing with secretory cargo to the plasma membrane.

Using a fluorescence pulse-chase labeling strategy to quantify cargo export out of the Golgi, Kory Hirschberg and George Patterson in my lab discovered that there is no lag or discrete transit time for cargo transport through the Golgi. Instead, incoming cargo molecules rapidly mixed with those already in the system and exited from partitioned domains at an exponential rate proportional to their total Golgi abundance (resembling radioactive decay; Patterson et al., 2008). This posed a challenge to cisternal progression in its classic form, which predicts that newly arrived cargo exhibits a lag or transit time before exiting the Golgi. Building on the idea that cholesterol-based increases in membrane thickness influence the subcellular distribution of membrane proteins relative to the length of their transmembrane domain (Bretschler and Munro, 1993) and that nonrandom lipid architecture is specifically geared to organize functionality within the bilayer (van Meer et al., 2008; Lingwood and Simons, 2010), we constructed a model of intra-Golgi transport to try to account for the new imaging data. In this model, cargo and Golgi-resident enzymes sort spatially due to their preferential affinity for different lipid domains in the Golgi. This, combined with the Golgi’s entry/exit fluxes, stack-like organization, and requirement of vesicular or tubule cargo transport across the stack (which prevents the system from becoming well mixed), results in a dynamic, self-organizing system. Simulation and experimental testing of this rapid partitioning model by Robert Phair and our lab showed that it produces all of the key characteristics of the Golgi apparatus, including polarized lipid and protein gradients, exponential cargo export kinetics, and cargo waves (Patterson et al., 2008).

The basic idea in this partitioning model is that compositional differences within the Golgi are maintained by bidirectional membrane flow. The striking live-cell imaging observations of Golgi dynamics in the yeast Saccharomyces cerevisiae by Ben Glick (Losev et al., 2006) and Aki Nakano (Matsuura-Tokita et al., 2006) showing that isolated Golgi elements undergo maturation fits with this idea (assuming that Golgi elements in yeast [Rambourg et al., 2001] resemble endosomal compartments [Sonnichsen et al., 2000], which are neither stacked nor have balanced bidirectional flow and so undergo continuous compositional change). Together these data suggest that the Golgi apparatus may not be a conventional organelle in the sense of being an autonomous entity comprised of stable components. Rather, the Golgi seems to function as a steady-state structure undergoing continuous outgrowth from and reorganization by the ER through bidirectional anterograde and retrograde trafficking (Altan-Bonnet et al., 2004).

In the future, we can expect many new surprises in this field as research uses newer fluorescent probes and imaging techniques to dissect spatial compartmentalization and temporal dynamics of molecules in the secretory pathway. Techniques such as fluorescence resonance energy transfer (FRET) and fluorescence correlation microscopy (FCS) should enable protein–protein interactions in this pathway to be spatially and temporally resolved, revealing potential interactions missed in classical biochemical assays that depend on large, isolatable samples for measurable interactions. Developments in fluorescent probes for better coimaging of proteins (and lipids) and for measuring and perturbing biochemical activities (i.e., GTP hydrolysis) will help in understanding how biochemical activities are organized to drive specific reactions at selected times and places. Finally, advances in superresolution microscopy, such as photoactivated localization microscopy (PALM) (Betzig et al., 2006), stochastic optical reconstruction microscopy (STORM; Rust et al., 2006), stimulated emission depletion microscopy (STED; Hell and Wichmann, 1994), and saturated structured illumination (Gustafsson, 2005), which provide spatial resolutions down to ∼20–60 nm, will enable optical examination of nanometer-scale phenomena of the Golgi and secretory pathway, including vesicle budding and fusion and tubule growth and tethering. These approaches, combined with correlative light-electron microscopy (Polishchuk et al., 2000) and biophysical techniques for monitoring membrane curvature, roughness, and tension, are likely to provide important new tools for solving many outstanding questions regarding the overall behavior and function of secretory transport.

From the foregoing, it should be clear that I am convinced that the development of new imaging technology has allowed the field to take a fresh look at the original paradigm of the secretory pathway. It has introduced data described by a vocabulary different from the standard static account of secretory organelles and the vision of discrete transport vesicles. This offers a potentially broader dynamic framework in which to situate the previous biochemical and genetic advances, with room for asking new questions. That, for me, is the excitement of doing science, and I eagerly await the results of research into these questions.

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Mitosis futures: the past is prologue

J. Richard McIntosh
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

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 microscopes. 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.

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...
Many kinds of small spindles, for example, algae (McIntosh et al., 1999; Grishchuk et al., 1995). 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 OsO4 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.

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 OsO4 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.

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 Atauilakhonov 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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Molecular motors: forty years of interdisciplinary research

James A. Spudich
Stanford University, Stanford, CA 94305

ABSTRACT A mere forty years ago it was unclear what motor molecules exist in cells that could be responsible for the variety of nonmuscle cell movements, including the “saltatory cytoplasmic particle movements” apparent by light microscopy. One wondered whether nonmuscle cells might have a myosin-like molecule, well known to investigators of muscle. Now we know that there are more than a hundred different molecular motors in eukaryotic cells that drive numerous biological processes and organize the cell’s dynamic city plan. Furthermore, in vitro motility assays, taken to the single-molecule level using techniques of physics, have allowed detailed characterization of the processes by which motor molecules transduce the chemical energy of ATP hydrolysis into mechanical movement. Molecular motor research is now at an exciting threshold of being able to enter into the realm of clinical applications.

It is an honor to be sharing the E. B. Wilson Medal with two friends and outstanding scientists, Dick McIntosh and Gary Borisy. We were all introduced to cytoskeletal research at about the same time. In my case, I was introduced to actin and myosin as a postdoctoral fellow at the MRC Laboratory of Molecular Biology in Cambridge, England, working with Hugh Huxley, on whose shoulders all molecular motor researchers stand. Hugh shared the 1983 E. B. Wilson Medal with Joseph Gall. My work with Hugh in 1969 and 1970 on the actin-tropomyosin-troponin-myosin muscle complex led us to postulate the steric blocking mechanism of calcium regulation of skeletal muscle (Spudich et al., 1972), more fully developed by Hugh elsewhere (Huxley, 1973).

After leaving Cambridge in 1971, I started my laboratory at the University of California, San Francisco, where I spent my first 6 years as a faculty member. In 1977, I moved to Stanford University. Over the years, I have had the privilege of working with the very best students, postdoctoral fellows, research associates, sabbatical visitors, and collaborators. This essay is a tribute to their contributions, rather than a minireview of the field. As it is, due to space limitations, I have had to leave out important contributions by many of my talented lab members, but a complete list of their contributions can be found on our laboratory website (http://spudlab.stanford.edu). This is a story of how our research evolved from 1971 to the present day. To put things in perspective, two key questions about molecular motors around that time were: How does myosin transduce the chemical energy of ATP hydrolysis into mechanical movement?, and What kind of molecular motors are in nonmuscle cells? I decided to focus on two goals: first, to develop quantitative in vitro motility assays for myosin movement on actin, essential for understanding energy transduction in this system; and second, to try to unravel the molecular basis of the myriad nonmuscle cell movements made apparent by light microscopy. During the first year of my assistant professorship, we searched for an ideal model system to study cell movements. We grew Neurospora, Saccharomycetes, Physarum, Dictostelium, Nitella, and other organisms totally unfamiliar to me at the time and searched for a myosin-like motor. Dictostelium proved to be perfect for this purpose. Margaret Clarke, one of my first postdoctoral fellows, identified a Dictostelium myosin with properties similar to those of conventional muscle myosin (Clarke and Spudich, 1974), and we then developed methods for visualizing the cytoskeleton in this and other nonmuscle cells (Clarke et al., 1975; Brown et al., 1976).
Regarding an in vitro motility assay, Dictyostelium, being a phagocytic organism, offered promise. Actin filaments were known to be in the cortex of nonmuscle cells, with their barbed ends at the cell membrane (Ishikawa et al., 1969; Schroeder, 1973). I fed Dictyostelium small polystyrene beads and isolated the phagocytic vesicles from cell lysates, and found that the vesicles had actin filaments emanating from the membrane-coated surfaces. With great excitement, I tried to establish in vitro motility of these vesicles along myosin-coated coverslips, but directed movements were not readily apparent, and a definitive in vitro motility assay would wait another decade.

THE DISCOVERY OF HOMOLOGOUS RECOMBINATION IN DICTYOSTELIUM
In the mid-1980s, I was considering shifting to a new model system, due to the lack of good genetic approaches for Dictyostelium. In collaboration with Leslie Leinwand, my student Arturo De Lozanne cloned the Dictyostelium myosin heavy chain gene. An interesting set of circumstances then led us to discover homologous recombination in this organism. Arturo disrupted the myosin II heavy chain gene (De Lozanne and Spudich, 1987) and Dietmar Manstein then managed its complete knockout (Manstein et al., 1989). These discoveries added a pivotal dimension to our research for the next couple of decades. We showed that the Dictyostelium nonmuscle myosin II is essential for cytokinesis, but not for cell migration. The latter was a surprise and a very important observation, since the prevailing dogma was that myosin II drove the forward movement of cells.

Over the following years, Hans Warrick, Tom Egelhoff, Jihong Zang, Sheri Moores, James Sabry, Wen Liang, Doug Robinson, and others in the lab characterized the structure–function relationship of Dictyostelium myosin II in cell division (e.g., Egelhoff et al., 1993; Sabry et al., 1997; Zang and Spudich, 1998; Robinson and Spudich, 2000). Importantly, having a null myosin II strain, Kathy Ruppel, Taro Uyeda, William Shih, Bruce Patterson, Coleen Murphy, and others were able to use specific mutations in the myosin II heavy chain gene to elucidate how this molecular motor works to transduce the chemical energy of ATP hydrolysis into mechanical movement (Uyeda et al., 1994, 1996; Patterson and Spudich, 1996; Ruppel and Spudich, 1996; Patterson et al., 1997; Shih et al., 2000; Murphy et al., 2001). Cathy Berlot, in collaboration with Peter Devreotes, explored the role of myosin II in Dictyostelium chemotaxis (Berlot et al., 1987), and Meg Titus and Holly Goodson discovered a number of other myosin isoforms in Dictyostelium and Saccharomyces and explored the roles of those isoforms in the biology of those cells (Titus et al., 1989; Goodson et al., 1996).

IN VITRO MOTILITY ASSAYS TAKEN TO THE SINGLE-MOLECULE LEVEL
To understand how myosin transduces the chemical energy of ATP hydrolysis into mechanical movement required us to achieve our second goal—develop quantitative in vitro motility assays for Dictyostelium. In 1979, Susan Brown managed to grow actin filaments on the surface of polystyrene beads with pointed ends out (Brown and Spudich, 1979); these were cleaner than the Dictyostelium phagocytic vesicles I had explored earlier, but we still failed to see convincing movement of these actin-coated beads in a myosin-coated surface assay. With Alan Weeds, a sabbatical visitor in 1982, we tried the reverse assay, to orient actin filaments on a surface by flow and watch myosin-coated beads move along them. Actin filaments were attached to a streptavidin-coated surface via barbed-end, bound, biotinylated severin. The filaments were oriented by flow and myosin-coated beads were expected to move upstream. Results were encouraging, but again not robust, probably due to poor orientation of the actin filaments. The lack of actin filament orientation was overcome in 1983, now with sabbatical visitor Mike Sheetz, when we used the well-ordered actin cables in Nitella to observe robust movement of myosin-coated beads, the first quantitative in vitro...
motility assay (Sheetz and Spudich, 1983). This assay allowed Tom Hynes, Steve Block, and Brian White to show that the N-terminal half of the myosin II motor was all that was needed for motility, ruling out some models of contraction (Hynes et al., 1987). The results from the Nitella assay led me and my new graduate student Steve Kron to push harder to get good orientation of actin filaments in the biotinylated severin filament assay, and in 1985 we published the first demonstration that purified actin and ATP are sufficient to support movement of myosin at rates consistent with the speeds of muscle contraction and other forms of cell motility (Spudich et al., 1985).

An even simpler in vitro motility assay harked back to the use of myosin-coated surfaces, but this time using Toshio Yanagida’s pivotal observation in Fumio Oosawa’s lab in 1984: individual actin filaments labeled with rhodamine-phalloidin are visible by fluorescence microscopy, and they show increased thermal bending motion of actin filaments in suspension in the presence of myosin and ATP (Yanagida et al., 1984). In 1985, Steve Kron showed robust directional gliding of individual rhodamine-phalloidin–labeled actin filaments along myosin-coated surfaces at velocities similar to those observed in muscle (Kron and Spudich, 1985, 1986). Shortly thereafter, Yoko Toyoshima and others in the lab established that the globular head, or subfragment 1 (S1) of the myosin molecule, is the motor domain (Toyoshima et al., 1987). Thereafter, all research on how the myosin family of molecular motors transduces the chemical energy of ATP hydrolysis into mechanical motion focused on the S1 head.

Fundamental questions remained, primarily focused on the step size that the myosin takes for each ATP hydrolysis. Various experiments from my lab suggested a step size of ~10 nm, while similar experiments from Yanagida’s lab reported values in excess of 50 nm (for review, see Altman and Spudich, 2005). A step size considerably larger than 10 nm would compel a model for how the actin-myosin interaction from the filament (for reviews, see Sellers and Veigel, 2006; Sweeney and Houdusse, 2007, 2010; Trybus, 2008; Spudich and Sivaramakrishnan, 2007). Other experiments by Ron Rock, Sarah Rice, Jeff Finer and sabbatical visitor Bob Simmons using a dual-beam laser trap they built in collaboration with Steve Chu (Simmons et al., 1993). Using the dual-beam laser trap allowed a single actin filament to be lowered onto a single myosin II molecule on the surface for direct observation of the step size (~10 nm) and the force (∼5 pN) produced during a single cycle of ATP hydrolysis (Finer et al., 1994).

NONMUSCLE PROCESSIVE MYOSINS DRIVE HOME THE SWINGING LEVER ARM HYPOTHESIS

The dual-beam laser-trap experiments led to a host of studies in our lab and others on nonmuscle myosins that are processive, meaning they undergo numerous steps along actin before completely disassociating from the filament (for reviews, see Sellers and Veigel, 2006; Sweeney and Houdusse, 2007, 2010; Trybus, 2008; Spudich and Sivaramakrishnan, 2010). Some of the important experiments from my laboratory using myosin V included the direct demonstration of myosin V processivity by Amit Mehta, Ron Rock, and Matthias Rief, in collaboration with Mark Mooseker and Richard Cheney (Mehta et al., 1999; Rief et al., 2000); the demonstration of single-molecule, high-resolution colocalization of two fluorescent probes (or SHREC) by Stirling Churchman (Churchman et al., 2005); and the observation of the behavior of the leading head as it searches for its actin binding site, ~36 nm in front of the rear head, by Alex Dunn (Dunn and Spudich, 2007). Other experiments by Ron Rock, Sarah Rice, and Tom Purcell, in collaboration with Lee Sweeney’s lab, showed processivity of myosin VI (Rock et al., 2001). This study resulted in the biggest challenge to the lever arm hypothesis; the step size was much too large for the presumed structure of the myosin VI dimer.

The resolution of this dilemma came partially from experiments by Zev Bryant and David Altman, who showed that the myosin VI lever arm swings a full ~180 degrees during its powerstroke (Bryant et al., 2007), and by Ben Spink and Sivaraj Sivaramakrishnan, who showed that the central part of the myosin VI tail is not a coiled-coil, but rather is a stable, relatively rigid, single α-helix, which could allow the dimer to straddle 36 nm along the actin filament (Spink et al., 2008). The processivity and large step size taken by these two myosin motors allowed detailed characterizations of how they work, and drove home the swinging lever arm hypothesis proposed by Hugh Huxley (Huxley, 1969).

WHERE DO WE GO FROM HERE?

There is still much to do in molecular motor research. For example, dynein is considerably more complex than kinesin and myosin, and its molecular basis of energy transduction is just beginning to be elucidated. With more than 50 kinesin types and 40 myosin types in most mammalian cells, the protein complexes that organize and regulate how these motors distribute the various cargoes in the cell into the detailed arrangements of the cell’s dynamic city plan remain mostly a mystery. My graduate students Dina Finan and Mandi Hartman recently identified a large number of proteins associated with just one of these motors, myosin VI (Finan et al., 2011). The field is also ready for emphasizing translational research involving the cytoskeleton (Malik et al., 2011). My laboratory now returns to muscle, this time cardiac muscle. Our goal is to use the many tools we have developed over the last several decades to understand the molecular basis of hypertrophic and dilated cardiomyopathy mutations that affect one out of 500 people in the general population and lead to heart failure and sudden cardiac death. I am fortunate to have a current laboratory group that is every bit as talented, energetic, and enthusiastic as in the previous years, and we look forward to a productive and stimulating period ahead.

ACKNOWLEDGMENTS

I am most thankful to all the talented students, postdoctoral fellows, research associates, and sabbatical visitors, past and present, who have made our journey into the field of molecular motors and the cytoskeleton so rewarding. I am totally immersed in our current studies on hypertrophic and dilated cardiomyopathies being carried out, in collaboration with Leslie Leinwand and her colleagues, by my currently highly talented group, including Kathy Ruppel, Shirley Sutton, Ruth Sommese, Mary Elting, Elizabeth Choe, Peiyi Chuan, Jingmin Sun, Kim Mortensen, Sadie Bartholomew, John Mercer, Tejas Gupta, and Suman Nag, all of whom I thank for joining this new effort. None of our work could have been done without the long-standing and generous support by the National Institutes of Health, as well as by grants from the Human Frontiers Science Program and the many agencies that provided fellowships to my students and postdoctoral fellows along the way.

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How we discovered fluorescent speckle microscopy

E. D. Salmona and Clare M. Watermanb

aDepartment of Biology, University of North Carolina, Chapel Hill, NC 27599; bCell Biology and Physiology Center, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892

ABSTRACT

Fluorescent speckle microscopy (FSM) is a method for measuring the movements and dynamic assembly of macromolecular assemblies such as cytoskeletal filaments (e.g., microtubules and actin) or focal adhesions within large arrays in living cells or in preparations in vitro. The discovery of the method depended on recognizing the importance of unexpected fluorescence images of microtubules obtained by time-lapse recording of vertebrate epithelial cells in culture. In cells that were injected with fluorescent tubulin at ∼10% of the cytosol pool, microtubules typically appeared as smooth threads with a nearly constant fluorescence intensity. One day, when an unusually low concentration of fluorescent tubulin was injected into cells, the images from a sensitive cooled charge-coupled detector camera showed microtubules with an unusual “speckled” appearance—there were fluorescent dots with variable intensity and spacing along the microtubules. A first thought was that the speckles were an artifact. With further thought, we surmised that the speckles could be telling us something about stochastic association of tubulin dimers with the growing end of a microtubule. Numerous experiments confirmed the latter hypothesis. Subsequently the method we call FSM has proven to be very valuable. The speckles turned out not to be a meaningless artifact, but rather a serendipitous find.

The discovery of the fluorescent speckle microscopy (FSM) technique depended on new advances in cameras with cooled charge-coupled device detectors (CCDs) and their application to fluorescence microscopy in cell biology in the early to mid-1990s. These cameras had significantly higher sensitivity (quantum efficiency), lower noise, and better spatial accuracy than the video cameras with image intensifiers that were commonly used at that time by cell biologists to obtain dynamic images of fluorescently labeled proteins in living cells. In 1996, Clare Waterman-Storer in the Salmon lab was examining how the polymerization and depolymerization of individual microtubules occurred near the leading edge of motile epithelial cells in culture (Waterman-Storer and Salmon, 1997). We were particularly interested in how the assembly of the actin filament cytoskeleton and its retrograde flow inward toward the cell center affected microtubule movement and assembly dynamics near the leading edge. To address this issue, Clare microinjected cells with purified tubulin dimers, the subunit protein of microtubules. The tubulin had been labeled with a red fluorescent fluorophore, X-rhodamine. She tried to inject enough X-rhodamine tubulin so that the labeled tubulin was ∼10% of the total cellular pool of tubulin subunits. After obtaining several time-lapse recordings with our cooled CCD camera of fluorescent microtubule assembly dynamics in her epithelial cell preparations, Clare left the microscope room and pulled Ted Salmon out of the lab to look at her time-lapse images. There was an unexpected feature that concerned her that had not been seen in previous publications in which images were recorded using an intensified video camera. In cells with high levels of injected fluorescent tubulin, the microtubules were brightly labeled, and fluorescence intensity appeared nearly constant along the lengths of microtubules (Figure 1A). However, in dim fluorescent cells containing low levels of injected tubulin, microtubules did not appear continuously labeled along their lengths but appeared as linear arrays of weakly fluorescent “speckles” that had the distribution expected for microtubules near the leading edge (Figure 1B). When Clare played back the time-lapse recording (Supplemental Video S1), it was apparent that the linear speckle arrays extended at their distal ends by adding new speckles with variable intensity and...
within cells. Indeed, Tim Mitchison noticed in time-lapse movies of individual fluorophores above the background autofluorescence doubted whether our imaging system was sensitive enough to see code" rather than widely spaced single fluorophores. We also higher than 0.01%, so the pattern was more like a "fluorescent bar because the concentration of fluorescent tubulin subunits was croinjected with very low amounts of fluorescent tubulin. However, (1995). This was achieved by including a very low concentration into an end during growth, the mean number of labeled tubulin dimers within a resolvable region along a microtubule is M = fN, whereas the SD (fluctuation in intensity) is given by \( fN(1 - f) \)^{0.5} \sim (M)^{0.5}. \) For \( f = 0.1 \) as in Figure 1A, \( M = 44 \) fluorophores in a resolvable region, whereas SD = 6.5, which is small compared with the mean. This explained the high contrast of speckles along microtubules in bright fluorescent cells injected with 10% labeled tubulin (Figure 1A). In contrast, for \( f = 0.01 \), as might have occurred in Figure 1B, \( M = 4.4 \) fluorophores and SD = 2.15, which is large compared with the mean. This explained the high contrast of speckles along microtubules in cells injected with 1% labeled tubulin or lower.

To prove this hypothesis correct, we had to do many tests (Waterman-Storer and Salmon, 1998, 1999; Waterman-Storer et al., 1998), which all were supportive (in particular, we had to satisfy the critical eye of Michael Caplow!). Our experimental results showed that fluorescent tubulin sediments as a 6S dimer in an analytical ultracentrifuge and thus was not forming oligomers. We showed that microtubules exhibit the expected fluorescent speckle patterns when assembled in vitro from pure tubulin and increasing concentrations of labeled dimer; speckle patterns are random and randomly change after microtubule shortening and regrowth; speckle contrast depends on the fraction of labeled tubulin dimer as predicted by the aforementioned equations; fluorescent tubulin speckle contrast does not depend on microtubule-associated proteins; the number of predicted fluorophores within a speckle at low fractions of labeled tubulin matches the number measured by steps in photo-bleaching; and, finally, the speckle intensity distributions along microtubules match predictions from computer simulations based on the foregoing model (Waterman-Storer and Salmon, 1999).

Our initial publications documenting the FSM technique showed how plus-end microtubule polymerization and depolymerization kinetics can be separated from motor-driven microtubule

FIGURE 1: Comparison of diffraction-limited fluorescent images recorded with a cooled CCD camera and 1.4-numerical aperture objective of microtubules in the lamella of a migrating newt lung epithelial cell injected with X-rhodamine–labeled tubulin. (A) Ten percent labeled tubulin and (B) 0.25% labeled tubulin in the cytosol. Scale bar, 10 µm. (Reproduced with permission from Waterman-Storer CM, Salmon ED (1999). Fluorescent speckle microscopy of microtubules: how low can you go? FASEB J 13(Suppl 2), S225–S230.)
translocation velocity for individual microtubules in interphase cytoplasm, the treadmilling of individual severed microtubule fragments near the leading edges of motile cells, and the two-dimensional kinetics of poleward flux of spindle microtubules in tissue cells and cytoplasmic extracts (Waterman-Storer and Salmon, 1997, 1998, 1999; Waterman-Storer et al., 1998). Low concentrations of microinjected fluorescent actin subunits were also used to demonstrate the advantages of FSM for analyzing the two-dimensional pattern of actin polymerization at the leading edge of migrating cells and the retrograde flow of actin filaments toward the cell center (Waterman-Storer et al., 1998).

Since its discovery, FSM has been used by numerous investigators of microtubule and actin polymerization and depolymerization dynamics and polymer motility in living cells and reconstituted preparations (Danuser and Waterman-Storer, 2006; Cameron et al., 2011) and has been applied to study the dynamics of other macromolecular ensembles, such as integrin-based focal adhesions (Hu et al., 2007). Indeed, a recent Google search on FSM turned up over 68,000 hits. The technology has been greatly enhanced by the work of Gaudenz Danuser and coworkers, who have developed sophisticated computer programs for automatically detecting speckles and measuring their translocation and lifetimes to convert observational FSM into quantitative FSM (Danuser and Waterman-Storer, 2006). This has revealed a multitude of dynamic information about microtubule and actin cytoskeletal dynamics and function during cell motility and microtubule assembly dynamics and poleward flux in mitotic spindles (Cameron et al., 2011). More recently, with the introduction of better cooled CCD cameras, including those with electron multiplication, measurement of polymer dynamics with single fluorophore speckles has become easier (e.g., Watanabe and Mitchison, 2002; Yang et al., 2007). Thus, our careful consideration of an imaging “artifact” serves as a powerful example of how paying attention to the results of even apparently failed experiments can lead to fortuitous discoveries.

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In the trenches: lessons for scientists from California’s Proposition 71 campaign

Lawrence S. B. Goldstein
Howard Hughes Medical Institute, Department of Cellular and Molecular Medicine, School of Medicine, and UCSD Stem Cell Program, University of California, San Diego, La Jolla, CA 92093

ABSTRACT I describe a number of valuable lessons I learned from participating in California’s Proposition 71 effort about the role that scientists and rigorous scientific advice can play in a public political process. I describe how scientists can provide valuable information and advice and how they can also gain a great deal from the experience that is valuable to a practicing research scientist. Finally, I argue that in the future, building similar broad coalitions to support biomedical and other areas of scientific research will be essential to protect publicly funded science. Thus, a key lesson from the Proposition 71 experience is that engagement of scientists with diverse nonscientific groups can make a big difference and that scientists must actively engage with the public in the future if we are to contribute robustly to the medical and economic health of our communities.

INTRODUCTION
From 2002–2004, I had the privilege of working with a diverse group of patient advocates, communications professionals, political consultants, scientists, and professional politicians to conceive, nurture, design, and secure the passage of California’s landmark Proposition 71 ballot initiative, which established the California Institute for Regenerative Medicine (CIRM). CIRM was designed to award $3 billion in competitive, peer-reviewed grant funds to stem cell research running from basic science to clinical application. The Proposition 71 ballot initiative that created CIRM passed with almost 60% of the voters supporting it, primarily because of the medical promise of stem cell research, the unique political pressures and ethical debate surrounding stem cell research in the United States, and the potential and recognized benefit that biomedical research brings to California’s biotechnology industry and economy. Much has been said and written about the merits and challenges of this landmark effort and its current function. I want to focus this essay, however, on a number of valuable lessons I learned about the role that scientists and rigorous scientific advice can play in the political process, in discussions with the public and patient advocates, and in the crucible of a high-visibility political and scientific initiative. In particular, I learned that, while the public political process can be messy, practicing research scientists not only can provide valuable information and advice, but can gain a great deal of valuable experience. I also learned that, while the world of science is not the only data-driven group, it nonetheless contributes a much longer-term perspective on issues than do other groups.

Finding a happy medium
“False hope!” “Don’t crush our hopes!” “Overhype!” “Too much pessimism!” I heard all of these conflicting statements and more from my patient advocate friends and colleagues during the Proposition 71 campaign. The lesson I learned is that one has to walk a fine line between too much pessimism, which some patient advocates will tell you has a negative impact, because it kills their hope, as
opposed to too much optimism, which contributes to the problem of overhyping or promising more than can be delivered in the time frame that you or someone else imagines. In short, I learned that I should paint a reasonable picture of what success would bring, while describing reasonable expectations and likely obstacles. For example, it is and was clear that success with stem cell approaches for type 1 diabetes will potentially bring complete insulin independence. But a number of important technical and safety problems will have to be solved on the path to achieving that goal, which leads to an uncertain time frame.

THE PROBLEM OF TIME
I have often been asked, both during the Proposition 71 process and since then, how long it will take a particular area of research to reach clinical application or, even harder to answer, “When will my wife’s, child’s, or parent’s disease be cured?” These are difficult, if not impossible, questions to answer, because the point of research is to find answers to unanswered questions, often with little accurate information available at the outset. The answers one gives thus require an explanation, not a number, since one cannot possibly give an accurate number. However, one can note correctly that expanding funding moves research more quickly. I also learned that it is appropriate to describe what success might look like, to talk about past experience with success in generating new therapies or discoveries, and to describe how broad portfolios of parallel opportunities will yield some that will proceed more quickly and some that will go more slowly. I also found analogy to be useful. In many settings, I would describe being a scientist in this (and other) fields as being like an explorer at the edge of a new continent, where I could see mountains and forests in the distance and knew it was likely that there were riches to be had. Thus I could argue it was worth proceeding and there would be great rewards, but predicting exactly how long it would take to yield results would be problematic. But I also learned that it is compelling to note that not starting a long journey leads to no benefit, or adds delay to what will be a long journey, no matter what. Nonetheless, the issue of time is hard and can also be difficult to communicate. Thus I also learned one can talk about short-term and long-term returns and describe what the process will look like. Finally, I learned that telling people that scientists are working as rapidly as possible to find better therapies is helpful. Many people respect this honesty and find it reassuring that the scientific community does care about trying to find therapies for people’s diseases, even if success will take time.

MEDIA AND SOUND BITES
The media and the communications industry are essential if scientists are to communicate with the public. In fact, the media is a megaphone that allows us to talk to more than just a few people at a time. In that context, the media is an essential part of how scientists work with other groups in political settings. But I often hear my colleagues say that “reporters never get it right” or that some reporter “didn’t quote them accurately” or that they “talked to a reporter for half an hour or more and they only used a one-sentence quote or a 15- to 20-s segment in the final radio or TV report.” My perspective and experience are different. First, my experience is that the vast majority of people I interacted with in the media are intelligent, hard-working professionals with little scientific background, who are nonetheless trying very hard to get the facts straight and to be as objective as possible. Second, my experience is that the point of a 20- or 30-min interview is that this is time a scientist can use to educate a reporter about the topic being covered, so the overall story is accurate, including the parts not directly based on the interview. Furthermore, my experience is that the journalist will be more likely to choose the right quote and/or get it right if the scientist has done a good job of providing an understandable explanation of the topic under discussion. To achieve this goal, however, requires that scientists learn to explain complex topics in plain everyday English with a minimum of jargon or Latinate language, which of course, is the same as good teaching. For example, I learned to say “blood-forming” stem cell instead of “hematopoietic stem cell” to nonscientific audiences.

THE PROBLEMS WITH TEAMWORK
Any coordinated action of a group with media and the public will inevitably require teamwork, as well as consolidation and discipline around shared messages. This can get tricky when the shared messages fail to agree with any one scientist’s view regarding correctness of information imparted or focus on the proper priorities. There is not an easy solution to this kind of problem. But if every member of a group chooses to broadcast his or her own version of a message, instead of uniting around common themes, what will emerge is cacophony, confusion, and likely failure. The question is how to balance scientific and personal integrity with teamwork, shared goals, and shared messages. My own solution was to work vigorously in private to ensure that the common message themes were rigorously correct, so that I, and my colleagues on the team, were always saying things that to the best of our knowledge were scientifically accurate and defensible. But I also chose to agree to focus on shared message points publicly, even if my own view was that these points were not as important scientifically as other messages. Similarly, I had to accept that the public at large and the scientific community sometimes use the same words differently. A case in point is the word “cure.” My personal and scientific view is that few diseases other than infectious diseases are ever truly “cured” and that what we offer are therapies that relieve, reduce, or manage a disease. In the public arena, however, what I regard as short- to medium-term therapies seem to be thought about as cures. In this case, my solution was to speak about therapies in my own interviews and public talks, but not to publicly debate the issues around the word “cure” with my own team. Thus my strategy was to be absolutely honest, while avoiding anarchy and confusion. In this situation, the crucial role of a scientist is to work as hard as possible privately to get the substance of the messages absolutely correct, and then to work with the team on promoting them in a coordinated and agreed-upon way.

APPLYING PROPOSITION 71 LESSONS TO OTHER INITIATIVES?
Building broad coalitions to support biomedical and other fields of scientific research is clearly an area where scientists, politicians, and other interested groups will have to work together more than ever to protect publicly funded science in the coming years. There is increasing scrutiny by politicians and the public, who want to know how the investment of their funds benefits them. Responding to that legitimate concern, and finding ways to continue to expand scientific research that supports healthy vigorous societies, will require scientists to be part of these teams of diverse interest groups. I can virtually guarantee from my experiences that decisions about funding and science policy made in the absence of scientific input will likely be unpalatable to scientists and ultimately not in the best interest of our broader societies. Thus a key lesson from the Proposition 71 experience is that engagement of scientists with diverse nonscientific groups can make a big difference and that scientists must actively engage with the public in the future if we are to contribute robustly to the medical and economic health of our communities.
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