A sustained passion for intracellular trafficking

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ABSTRACT I am honored to be the first recipient of the Women in Cell Biology Sustained Excellence in Research Award. Since my graduate school days, I have enjoyed being part of a stimulating scientific community the American Society for Cell Biology embodies. Having found myself largely by accident in a career that I find deeply enjoyable and fulfilling, I hope here to convey a sense that one need not have a "grand plan" to have a successful life in science. Simply following one's interests and passions can sustain a career, even though it may involve some migration.

AN EARLY MOVE SOUTH—WAY SOUTH

Although I was born in Princeton, New Jersey, I grew up half a world away in Melbourne, Australia. This was the direct result of the peculiarities of the academic job market, even in the mid-1970s. My father was a mathematician at Princeton's Institute for Advanced Studies and was lured to the antipodes by an attractive offer from the University of Melbourne. I'm sure my parents expected to come back to the United States in due course, but the relaxed Australian lifestyle suited our family, and we settled permanently in Melbourne. Because I was only three years old when we moved, I developed a deceptive Australian accent that belies my New Jersey heritage!

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At high school and university (both publicly funded), I followed a meandering path from biology to mathematics to psychology and back to biology. The broad education that I received as an undergraduate at the University of Melbourne, with an emphasis on systematics and evolution, built a solid foundation that frames the more molecular and mechanistic questions I ask today. Like many others, my laboratory experience was limited to prescribed classes, so my first summer of "real" research at the end of my third year was a revelation. I became fascinated by the elegant beauty of the single-celled algae that were the focus of study in Rick Wetherbee's lab in the Botany School. I worked with a postdoctoral fellow, Jan Lind, to characterize the extracellular glycoproteins these organisms use as cellular glues (Ludwig *et al.*, 1996). I continued this work as an "honours" student, which in the Australian system is an optional fourth year of university that allows a full year of dedicated bench research. By the end of my honors year, Rick had secured a grant from the U.S. Office of Naval Research to investigate the marine diatoms that are the primary drivers of biofouling on ship hulls. This allowed me to work as a research assistant for a year, while I considered what I wanted to do next with my life, thinking for the first time that one could actually make a living doing this fun research thing.

During my time in Rick's lab I started to appreciate the problem of how cells move macromolecules (like those secreted glycoproteins) around in very precise ways. With this interest in intracellular protein traffic in mind, I went looking for a lab that would further my training and afford some scientific independence. I was fortunate to join Marilyn Anderson in 1995 as she moved from the University of Melbourne to La Trobe University (located in a suburb of Melbourne). I joined her small group to initiate a new project investigating the delivery of a plant defense protein, NaPI, to the vacuole in tobacco cells. We hadn't been at La Trobe long when Trevor Lithgow joined the department, introducing me to the benefits of yeast as a model organism. Under the joint supervision of Marilyn and Trevor, I defined the vacuolar sorting signal of NaPI and identified a candidate receptor

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that likely diverts the protein from the default pathway of secretion to an endosome-like prevacuolar compartment (Miller *et al.*, 1999). Aside from the superb scientific training I received from my advisors, graduate school was formative in other ways. Most importantly, I forged lasting friendships with my peers, including my now-husband, Marcus Lee (a fellow graduate student in Marilyn's lab), that enriched my life both at the bench and beyond.

After four years in Marilyn's lab, it was time to move on and find the next step in my own path. Inspired by both of my mentors' fulfilling overseas postdocs (Marilyn at Cold Spring Harbor and Trevor at the Biozentrum in Basel), I sought out postdoctoral opportunities with those whose work I admired. In retrospect, my approach was very naive, and I am fortunate that I was even invited to interview with so many great labs, coming as I did from a somewhat obscure university in a distant land with (as yet) no publications to my name. Nonetheless, both Marcus and I managed to find excellent opportunities not only in the same city, but in the same lab, this time working with Randy Schekman. So, we gave away our furniture, packed our suitcases, and headed to Berkeley, California.

LIVING THE CALIFORNIA DREAM

Joining the Schekman lab was another revelation. I was suddenly surrounded by a sizable cadre of smart, driven colleagues, both postdoctoral fellows and graduate students, all of whom focused on intellectual problems that fascinated me. The lab was well-funded, and we all had enormous freedom to pursue our own questions, guided always by Randy's own powerful and creative intellect. After a false start on a project to develop a new in vitro reconstitution assay, Randy suggested I look into the molecular function of one of the coat proteins that drives vesicle formation from the endoplasmic reticulum (Barlowe *et al.*, 1994). Characterization of the COPII coat was pioneered in Randy's lab, and we had in hand many genetic tools and in vitro assays that facilitated this new project. I used a combination of yeast genetics and biochemistry to delineate the COPII subunit Sec24 as the primary cargo-binding component of the coat (Miller *et al.*, 2002). My findings dovetailed nicely with x-ray crystal structures that Elena Mossessova solved in Jonathan Goldberg's lab, and we ended up publishing our findings in back-to-back papers (Miller *et al.*, 2003; Mossessova *et al.*, 2003). The panel of mutants that I generated in Randy's lab still provides interesting new leads (Kung *et al.*, 2012), and we continue to use the powerful approaches of yeast genetics and biochemical assays that mutually inform each other. In retrospect, my postdoctoral years seem like glorious, carefree days when I had the time, resources, and stimulating environment to simply do the very best research I could. Again, a group of like-minded colleagues, especially David Madden, Per Malkus, and Raphael Valdivia, greatly enriched both my bench and leisure lives. Yet, once again, it all too soon became time to move on.

Although the obvious "next step" after a postdoctoral fellowship is an independent faculty position (at least here in the United States), I have to confess that I didn't necessarily see myself in that role at the end of my postdoc. I didn't have a long-term career plan, having instead simply been driven by the joy that I derived from doing research and the desire to keep having fun. Nonetheless, I had to find a new position after several years in Randy's lab. So I went on the job market, looking mainly in larger cities that would also afford opportunities for Marcus, who wanted to change fields and explore protein trafficking in the malarial parasite. Our "two-body problem" was solved in New York City, with Marcus joining a malaria lab on a second postdoctoral fellowship, while I joined the Department of Biological Sciences at Columbia University. Fortunately for me, it turns out that running a lab is also a lot of fun, even if I don't get to do as much bench work as I'd like.

BRIGHT LIGHTS, BIG CITY, SMALL LAB

My lab continues to explore the molecular mechanisms of vesicle formation from the endoplasmic reticulum, focusing on how protein folding influences this process. It has long been known that misfolded or improperly assembled proteins are not captured into COPII vesicles, but the mechanism by which this quality-control checkpoint works remains unclear. This problem is central to a variety of human diseases, most notably cystic fibrosis, which is often caused by mutations in a chloride channel that cause the protein to misfold. We aimed to use yeast as a model system to probe the cellular pathways that contribute to biogenesis of a related yeast protein, Yor1 (Pagant *et al.*, 2007). I was fortunate to receive pilot funding and a subsequent full grant from the Cystic Fibrosis Foundation, who were persuaded that we could successfully model a human disease in a genetically tractable system. My association with the Cystic Fibrosis Foundation led directly to a fruitful collaboration with John Hartman, an expert in quantitative genomics, who introduced us to the art of high-throughput screening (Louie *et al.*, 2012). This approach has yielded a jackpot of mutants that we continue to characterize mechanistically using our biochemical assays.

It goes without saying that the success of my lab is entirely the result of the fantastic students and postdocs with whom I have worked. I am grateful to my first crop of graduate students, Ray Louie, Leslie Kung, and Mariana Dorrington, who took a chance on a green principal investigator and joined my lab when we were still unpacking boxes. One of my great mentors at Columbia, Marty Chalfie, advised me that initially I would be my own best postdoc. Happily for me, this was not entirely true, as I managed to recruit a former colleague from the Schekman lab, Silvere Pagant, who remains in the lab to this day as a senior research associate, leading our efforts on quality control of Yor1. The work of two talented postdocs, Alenka Copic and Cath Latham, opened up a new view of the problem of quality control, turning our focus to the physical properties of the vesicles themselves (Copic *et al.*, 2012). Another dedicated student, Jenn D'Arcangelo, is well underway with her efforts to follow up on our new model, which proposes that the protein quality-control "checkpoint" is in fact a stochastic product of cargo occupancy and vesicle architecture. There are clearly many exciting discoveries for us to make in the coming years.

Along with the debt of gratitude I owe my lab members, I am also extremely thankful for the support and encouragement of many mentors over the years. In addition to my research advisors, I deeply appreciate the support of my former department chairs and perennial cheerleaders: Carol Prives, Mike Sheetz, Marty Chalfie, and Stuart Firestein. Respected colleagues Bill Wickner, Frances Brodsky, Lois Weisman, and Susan Michaelis are scientific supporters whose enthusiasm for my work is truly gratifying. Indeed, I am grateful to the many talented colleagues who study intracellular membrane traffic and contribute to the supportive nature of the field as a whole. My impression is that this collegial community is, like I am, driven by a passion for science and excited by new discoveries, whatever their sources. Finally, whatever success I have achieved professionally would not be possible without the support—both intellectually and personally—of my husband, who is an equal partner in raising our son and has made career sacrifices of his own to afford me the opportunities I've had.

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