Spatial and temporal impacts on a career in science

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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
allosteric models. 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 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, the first three we identified shared unusual 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 plcl1 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 in 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 Straw 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...
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. Coincidently, 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.

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