From junior to senior: advice from the benefit of 20/20 hindsight

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ABSTRACT **As the first recipient of both the Women in Cell Biology Junior and Senior Awards, I look back to identify key components that have provided the foundation for my successful research career. In retrospect, the three most important building blocks have been: identifying and pursing important problems; attracting and mentoring talented postdoctoral fellows and students; and establishing and nurturing strong collaborations.**

In 1990, I was honored to receive the Women in Cell Biology (WICB) Junior Award, which recognized my "significant potential" for scientific contributions. Twenty-four years later (where did the time go?), presumably having met those high expectations, I am once again honored to receive the WICB Senior Award. Being the first recipient of both awards has prompted me to look back, consider, and share what worked, what did not, and what lessons I have learned in the process. Thus, with the benefit of 20/20 hindsight, I offer the following advice to this and future years' WICB Junior Award recipients.

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IDENTIFY AN IMPORTANT PROBLEM AND PURSUE LONG-TERM GOALS

First and foremost, you must identify a good problem on which to focus your research program. You must be passionate about the subject. You should be excited to read new papers and reviews as soon as they appear, and to discuss their merits and shortcomings and the new experiments they suggest with anyone who will listen. You need to become a fanatic—an expert! You should be able to identify many unanswered questions, some immediately addressable and others that must await new information and new technologies that you can only begin to imagine. "I wish I could …" That is, you must become obsessed with knowing the details. But, the problem must also be one for which you can balance this obsession for details with a vision of the infinite. "What if …?" "If so, then this could mean …!" Pick a problem that you can address from a new perspective and/or by applying new methodologies or experimental systems that reflect your unique skill set and training background.

I was lucky and found my passion early. When I began my graduate studies in 1980, I chose to study clathrin-mediated endocytosis (CME), still the subject of my research program. I had first encountered coated vesicle–mediated endocytosis during a cytology class while studying cell biology at the University of British Columbia. Viewing the spectacular electron micrographs of Roth and Porter showing uptake of yolk proteins by coated pits and vesicles in mosquito embryos after their mother's blood meal (Roth and Porter, 1964) and those of Heuser and Reese of the same structures recycling synaptic vesicles after excitation of a frog neuromuscular junction (Heuser and Reese, 1973) piqued my curiosity and imagination. Barbara Pearse had recently purified coated vesicles from porcine brain and identified clathrin as their major coat constituent (Pearse, 1975, 1976). A slew of papers had just appeared showing that ferritin- (Anderson *et al.*, 1977) or ^{125}I-labeled (Gorden *et al.*, 1978) ligands and their receptors were concentrated in clathrin-coated pits and vesicles (CCVs) for efficient internalization. I was swept up in this wave of exciting new discoveries. Moreover, working on my honors thesis project in the lab of Pieter Cullis, who

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Abbreviations used: CCV, clathrin-coated pits and vesicles; CME, clathrin-mediated endocytosis; WICB, Women in Cell Biology.

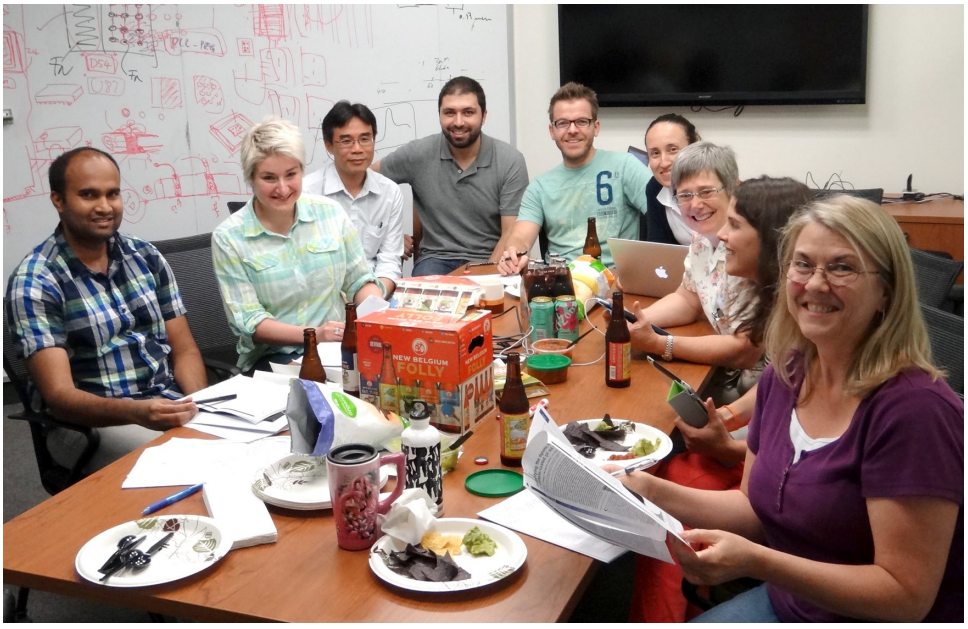

FIGURE 1: Schmid (third from right) and current lab members at journal club actively discussing the newest papers, their merits and shortcomings, and the new experiments they suggest.

studied nonbilayer phospholipids and their role in membrane dynamics, I wondered which proteins worked together with clathrin to build this elegant cellular machinery and how it could work to deform and pinch off a small piece of the membrane while still maintaining its critical barrier function. There were so many unanswered questions.

At about the same time, I attended a seminar and had lunch with a young assistant professor, James Rothman, who had just started his lab at Stanford University. He reported their as-yet-unpublished, early progress toward the first cell-free reconstitution of a vesicular trafficking event (Fries and Rothman, 1980). This was exciting, as the tools were becoming available to measure and understand vesicular transport. Thus I began my graduate studies in Jim's lab with the goal of reconstituting CME.

I quickly learned that inside every big problem are a lot of little problems. In the Biochemistry Department at Stanford University, founded and inspired by Arthur Kornberg, reconstituting complex biological reactions from purified components was almost expected. However, the application of biochemical fractionation and reconstitution to membrane trafficking events was in its infancy. Of course, I was not successful in reconstituting CCV formation during my 4 years at Stanford and instead answered a much simpler problem: given that clathrin could spontaneously assemble into "empty cages" (Woodward and Roth, 1978), we reasoned that energy must be required to disassemble clathrin coats with the help of some yet undiscovered uncoating enzyme. My colleagues (David Schlossman and Bill Braell) and I established sedimentation assays for uncoating and used these to purify and characterize the uncoating ATPase now known to be hsc70 (Braell *et al.*, 1984; Schlossman *et al.*, 1984; Schmid *et al.*, 1984; Rothman and Schmid, 1986).

It became clear that to solve the bigger problem of CME, I would need more skills as a cell biologist. And so I moved to Yale to pursue studies among the pioneers of membrane trafficking, George Palade, Marilyn Farquhar, Jim Jamieson, and another young assistant professor just starting his lab, Ira Mellman. Ira and Ari Helenius had recently discovered endosomes and were developing new methods of subcellular fractionation to study them. Here was an opportunity to apply my newfound skills as a biochemist and to be immersed in cell biology. We were able to purify and identify biochemically and functionally distinct early and late endosomes (Schmid *et al.*, 1988).

As an assistant professor at the Scripps Research Institute, I returned my focus to the reconstitution of CME. Many talented postdocs contributed to our efforts, allowing us to reconstitute and study CME in perforated cells (Schmid and Smythe, 1991; Carter *et al.*, 1993) and from isolated plasma membrane sheets (Miwako *et al.*, 2003). These studies also led us to focus on the GTPase dynamin, which we eventually showed not only functions as the minimal fission machinery (Pucadyil and Schmid, 2008; Shnyrova *et al.*, 2013), but also regulates early, rate-limiting steps in CME (Sever *et al.*, 1999, 2000; Aguet *et al.*, 2013). Along the way toward our goal of reconstituting CCV formation from its minimum components, we also discovered important two-way links between CME and signaling (Lamaze *et al.*, 1996; Vieira *et al.*, 1996; Conner and Schmid, 2002). Thus it became clear that rather than defining the minimal components, which were later shown to be clathrin, a membrane adaptor, and dynamin (Dannhauser and Ungewickell, 2012), we needed to understand the complexity and regulation of CME. We needed to define the "maximum" components required for this physiologically critical process. This goal could only be accomplished in living cells: a goal now attainable by technological advances, such as green fluorescent protein, RNA interference, total internal reflection fluorescence microscopy, computer-aided image analysis, genome-editing, and others that did not exist in 1980.

Almost 35 years after choosing to study CME, the process continues to fascinate me, and our studies continue to reveal new concepts, such as the existence of an "endocytic checkpoint" (Loerke *et al.*, 2009; Aguet *et al.*, 2013), and unexpected twists, such as the ability of specific cargo molecules to "fine-tune" and "customize" the endocytic machinery (Lamaze *et al.*, 1993; Lamaze and Schmid, 1995; Liu *et al.*, 2010; Mettlen *et al.*, 2010). My enthusiasm for reading the newest papers and discussing their merits and shortcomings and the new experiments they suggest has never diminished.

BE A GOOD MENTOR

As a new assistant professor, your skills at the bench and your direct eyes on the results and incongruities will be critical for your success. Stay active at the bench for as long as possible! However, as your lab grows and begins to incorporate new technologies, your role will change. You will need to be effective in facilitating the work of others, rather than performing experiments yourself.

Set high standards for membership in your lab and be explicit about your expectations for effort and attitude. Value every member and realize that each has his or her own strengths, weaknesses, aspirations, and needs. Watch and listen to discover what these are. Some will be well-trained, extremely independent, and ambitious—challenge them to be disciplined, goal-oriented, risk-takers and to mentor others. Some will require closer supervision and more frequent direction until they gain the skills needed for independence. Don't make them struggle alone. Instead, work with them more closely or pair them up with more senior lab members to efficiently teach them the skills they need for success. Others, with your help, will discover that they'd rather be doing something else. Help them, as quickly as possible, to find their passion and new opportunities to pursue it. If they are in the wrong place and lack motivation, they could create negative feedback that could impact overall lab morale.

When I started my lab, I assumed that all postdocs had their own good ideas and ability to execute them and that, like me, they needed/wanted minimum oversight from their mentors. I treated all my postdocs in the same way and each worked independently on his or her own projects. We were a small lab of two postdocs and one technician working on four different projects. It was a disaster! While some succeeded, others floundered and became frustrated and demotivated. Imposing more direction later on was difficult. Today, every new member of my lab begins by working with a more senior member on a well-defined project. The senior member learns mentorship skills and, in exchange for training a new lab member, his or her project advances more quickly. The junior member quickly learns new skills and experiences early success. Independent projects emerge at variable times, as each individual develops the ideas necessary to branch out. My lab works and succeeds as a team.

Recognize and reward the individual accomplishments of your postdocs and students, even (or especially) within a team. Then actively help them to transition to the next stages of their own careers. Their success will create positive feedback that motivates current members and attracts talented new members to join your lab.

FIND AND NURTURE GOOD COLLABORATORS

Effectively tackling big and important questions will require many different technologies and approaches. Pursuing your results will take you down unfamiliar paths. Do not fear them. There is no reason to stop and pull back or to move slowly forward, hobbled by inexperience. Science is increasingly interdisciplinary, but individual scientists can't possibly be. Seek out the experts whose approach, when applied to your problem, will be mutually beneficial, allowing you both to accomplish an important objective that neither could accomplish alone. Make sure you share credit, engage in honest and open communication, and build a relationship based on trust and mutual respect.

I have benefited from outstanding collaborators throughout my career, starting with the already-mentioned David Schlossman and Bill Braell, postdocs with Jim Rothman, who taught me biochemistry and enzymology. With their help, I got a quick start as a graduate student and was able to publish eight primary papers and to complete my Ph.D. training in 4 years. At Yale, I teamed up with Renate Fuchs, a skilled and knowledgeable physiologist who could measure ion transport across endosomal membranes. I worked the early shift, preparing endosomal fractions in the mornings, and Renate would take over in the afternoons and evenings to characterize their transport activities. Together, we published three papers in 2 years and, more importantly, developed a lasting friendship. To understand dynamin function, I have collaborated with brilliant physicists (Vadim Frolov and Josh Zimmerberg) and talented structural biologists (Jenny Hinshaw, Ron Milligan, Josh Chappie, and Fred Dyda) with great success. For the past 10 years, I have enjoyed a close collaboration with Gaudenz Danuser, an engineer and mathematician, and his talented lab members who have helped us to develop and analyze live-cell assays for CME. These collaborators have pushed me to accomplish goals I could not have reached alone and to ask questions in new ways and from new perspectives. They too have become valued friends.

By far my most successful and rewarding collaboration has been with my husband, Bill Balch, whom I met at Stanford, while he was a postdoc with Rothman. While we have never published together, Bill has been an important advocate, critic, source of support, and sounding board throughout my career. We have collaborated in raising two outstanding young adults, Jeremy, who began medical school at University of Michigan this fall, and Katherine, a composer (www.katherinebalch.com) studying at Yale. Both are happy, accomplished, and successfully following their own passions. Thus my last piece of advice to current and future Junior Award recipients is to enjoy and value your families and loved ones, as these relationships provide the support needed to persevere when times are tough, to believe in yourself, to take risks, and to accomplish your goals.

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