What does it take to get the job done?

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ABSTRACT **I am extremely honored to be the recipient of the 2015 Women in Cell Biology Junior Award. When I reflect on my journey in science, many great people and memorable experiences come to mind. Some of these encounters were truly career-defining moments. Others provided priceless lessons. In this essay, I recount some of the moments and experiences that influenced my scientific trajectory with the hope that they may inspire others.**

THE BIG QUESTION

It was a tense day in late fall of 2011. I was going through my first site visit at the National Institutes of Health (NIH), a comprehensive review that would define my scientific career. My three-year-old lab had published a significant paper on mechanisms that shape bone morphogenetic protein (BMP) morphogen gradients and control early patterning (Peluso *et al.*, 2011), and we had discovered *Drosophila* Neto, an obligatory auxiliary subunit for glutamate receptors. The question filled the room: "The BMP/transforming growth factor β (TGF-β) field has been tackled for quite some time by many laboratories. You propose to work on these pathways. What are you hoping to bring that is new to the field and how?" It was a very fair and simple question coming from one of the most accomplished and clear thinkers in cellular and developmental biology today, Eric Wieschaus. In a few words he crystallized the key issue any junior principal investigator should think hard about: What do I bring that is new to science?

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In my lab, I address fundamental issues of cellular communication using a genetic system and a unique set of powerful, multidisciplinary approaches for solving problems. This scientific strength was shaped by my diverse background and experiences and is fueled by a need to understand the molecular mechanisms underlying biological phenomena. I am drawn to long-standing mysteries in the field, and my first impulse is to imagine what kind of molecule/function(s) could fill the missing link. What does it take to get the job done? In my search for answers, I reach across disciplines and communicate with diverse experts, from cell biologists to neuroscientists and computational biologists. I gather comprehensive knowledge of the system and the phenomena to understand the "job" and to describe it in molecular terms. Then I use biochemistry and structure–function insights to envision possibilities and formulate a testable hypothesis.

"What does it take to get the job done?" not only describes the thinking process in my lab but also captures our mode of operation. We will do everything possible to answer the next question, whether it means perfecting our skills, inventing tools, bringing new technologies to the lab, or establishing relevant collaborations.

FINDING ONE'S PASSION

It takes scholarly work and courage to break new ground, but first one must find his/her passion. I knew early on that I wanted to be a scientist. At first I thought I would be a mathematician. But, because I was a girl, I was persuaded to look elsewhere. I chose chemistry and genetics and decided to study biochemistry. This was one of the best decisions in my life and was entirely mine. I was 17 years old, and I literally took three days to think hard and consider everything that I was passionate about: logical thinking,

DOI:10.1091/mbc.E15-06-0428
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Abbreviations used: BMP, bone morphogenetic protein; iGluRs, glutamate-gated ion channels; NIH, National Institutes of Health; NMJ, neuromuscular junction; pMad, phosphorylated Smad; TGF-β, transforming growth factor β.

genetics, molecules, living creatures, and solving puzzles. Within a year I moved to Bucharest to study biochemistry.

My first encounter with cell biology was self-driven. The biochemistry curriculum at the University of Bucharest was very heavy on chemistry, especially in the first years. I was searching for ways to put things in perspective. A friend recommended a cell biology textbook adapted from *Molecular Biology of the Cell* by Bruce Alberts and colleagues (Alberts *et al.*, 2014). I bought it and started to read. I ended up immersed in it, losing track of time. After graduation, I was drawn toward the Institute for Cellular Biology and Pathology in Bucharest, the place with the best cell biology research in Romania.

Many scientists have turning points in their careers, when they find out what they really want to do in science. Mine was when I met George Emil Palade, who came to give a seminar at the institute. He made a comment that intrigued me: he considered his work on protein regulation by phosphorylation even more important than the discovery of ribosomes. From the perspective of a freshly graduated scientist, the body of work that brought Palade the Nobel Prize was simply monumental. How could anything be more important than that? What was so significant about protein phosphorylation, and why was this a key finding in biology? I was enthralled. This was my introduction to cellular signaling. This moment triggered a fascination with signaling and macromolecular complexes that I have to this day.

THE JOURNEY

To work on signaling, I joined Dan Kosman's laboratory at the State University of New York at Buffalo, which focused on cellular mechanisms to acquire and metabolize iron. The first winter in Buffalo was unforgettable: braving my way through record snowfall, I was taking terrific courses, such enzyme kinetics taught by Cecile Pickart and molecular biology taught by Ed Niles. For my PhD thesis, I studied how a simple eukaryotic cell, the budding yeast, senses and responds to the levels of copper and iron in its environment. It was a time of intense discoveries in the field. Dan Kosman encouraged us to go to meetings and grasp the latest news. I learned a lot from seeing how he discussed our findings and interacted with his competitors. He is a rigorous scientist and a master strategist.

For postdoctoral training, I joined Mike O'Connor's laboratory at the University of Minnesota to work on signaling by BMP/TGF-β factors during *Drosophila* development. Up to that point, I did not know anything about development, but it seemed to be the best place to probe for the biological relevance of signaling. It was a daunting yet exhilarating experience to dive into a model system with such prominent history in genetics and development. I went back to take courses, including *Drosophila* genetics at the Cold Spring Harbor Laboratory and developmental biology taught by Ann Rougvie at the University of Minnesota. It was a steep learning curve, but Mike O'Connor's laboratory and the Developmental Biology Center offered an interactive and stimulating environment. The lab was extremely productive and full of fantastic people. New discoveries were coming in big leaps rather than in a linear progression. We all had different individual projects, and it seemed that each story tackled another field. I was always mesmerized by Mike O'Connor's ease at entering a new field and making an important discovery. The lab was in the middle of a vibrant fly community, including Tom Hays, Jeff Simon, Tom Newfeld, and Hiroshi Nakato. Fly stocks and good ideas were moving freely. I learned to do microinjections in *Xenopus* embryos in Jamie Lohr's lab. And I tested the axon guidance defects of a *Caenorhabditis elegans* mutant in Lishia Chen's lab. During these years, I described several molecular mechanisms that shape morphogen gradients during patterning (Serpe *et al.*, 2005, 2008; Umulis *et al.*, 2006). I formed a long-term collaboration with David Umulis (now at Purdue University), who introduced me to computational biology. And I characterized a TGF-β pathway required for motor neuron axon guidance and formation of neural circuitry (Serpe and O'Connor, 2006). This last bit was the beginning of a new chapter, neural development, which was about to captivate me for the rest of my career.

THE UNIT ON CELLULAR COMMUNICATION

The big surprise in starting a new lab is the loneliness that comes with its beginning. Fresh from a lab buzzing with people and experiments, you are faced with an empty space. And it is up to you to bring it to life and make things happen. The way I looked at it was that it another new ground to break, which takes scholarly work and courage. This time I bought the book *At the Helm* by Kathy Barker (Barker, 2010), and I took management courses. Courage was asking for help.

My corner of the NIH is full of excellent scientists who challenge themselves and push the boundaries. In this environment, I found many answers and important role models. I learned from Alan Hinnebusch to set high standards and from Mary Dasso to become an effective mentor and woman scientist. I learned to distill questions in neurobiology from the late Howard Nash, who initiated and mentored the *Drosophila* neurobiology interest group. We regularly brainstorm on neurons and circuits in joint lab meetings with Chi-Hon Lee and Ed Giniger.

The fly neuromuscular junction (NMJ) has been a powerful genetic system to study synapse development. The easily accessible synaptic structures were well described, the subunits that form the glutamate-gated ion channels (iGluRs) were known and relatively well characterized, and dynamic studies have captured growing synapses (DiAntonio, 2006; Thomas and Sigrist, 2012). But a nagging problem was holding up the field: the mechanisms controlling the synaptic recruitment of iGluRs remained unknown for decades. Studies on iGluR C-tails, which provide rich regulation for these receptors in other systems, brought marginal progress in flies. We found an interesting transmembrane molecule, Neto (Neuropillin and Tolloid-like protein), with a spectacular phenotype: *neto*^{null} embryos are completely paralyzed and cannot hatch into the larval stages. This is reminiscent of the phenotype seen with the loss of iGluRs and lack of functional NMJ. In fact, we found that Neto and iGluRs form complexes and depend on each other for trafficking and clustering at synaptic sites (Kim *et al.*, 2012). Neto is an obligatory auxiliary protein for the fly NMJ iGluRs. The discovery of Neto provides an entry point to address key questions in iGluR cell biology and to start dissecting the individual steps of iGluR assembly, surface delivery, trafficking, and stabilization at synaptic locations; function; and postsynaptic composition. We have already found that Neto engages in intracellular and extracellular interactions that recruit postsynaptic components and stabilize iGluRs at synaptic locations (Kim *et al.*, 2015; Ramos *et al.*, 2015). In collaboration with Mark Mayer, we have achieved the long-sought functional reconstitution of NMJ iGluRs in heterologous systems and showed that Neto modulates the functioning of iGluRs but not their assembly or surface delivery (Han *et al.*, 2015).

Neto also appears to be at the center of transsynaptic complexes that monitor the synapse activity status and relay this information to the presynaptic BMP signaling pathway (Sulkowski *et al.*, 2014). At the fly NMJ, BMP signaling triggers accumulation of phosphorylated Smad (pMad) in motor neuron nuclei and at synaptic terminals. Nuclear pMad controls gene expression and promotes NMJ growth; the role of synaptic pMad eluded the field for more than a decade. We found that synaptic pMad is part of a completely new BMP pathway that is genetically distinguishable from all known

BMP signaling cascades. This novel pathway does not contribute to the NMJ growth and instead appears to set up a positive-feedback loop that modulates synapse maturation as a function of synapse activity. Thus, BMPs may monitor synapse activity and coordinate it with synapse growth and maturation.

THE PEOPLE

When I reflect on how this all came together, many great people and mentors come to mind. First are the people in my lab, a team of talented, budding scientists whom I have been privileged to guide in exciting endeavors. Their success is critical to me from the moment they join my lab to, I suspect, long after they leave. Second are my colleagues—and here I hit the jackpot. Ours can be a stressful profession, particularly at the beginning of a new lab. But if you have colleagues like I do, incredibly accomplished scientists with big hearts and a deep commitment to mentoring their juniors, you could survive even when the building is falling on you. I am especially grateful to Alan Hinnebusch, Mary Dasso, and Chi-Hon Lee for their continuous guidance and support. They believed in me from the beginning, showed confidence in my decisions, and helped me keep the course. I owe a great deal to our collaborators, who taught us exciting new things and enriched our science. Bing Zhang completed the first physiology recordings for our mutants, and then helped set up our own rig. Without Mark Mayer, we could not have done the iGluR functional reconstitution and structural studies. We learned to harness the power of superresolution imaging in Jennifer Lippincott-Schwartz's laboratory.

In science, as in life, one could never return the help and support received along the way. All we can hope is to do the same in the future. When my trainees go on the job market, I paraphrase for them the advice I received: In this profession there will be many worries, good and bad. What BMPs do in a particular cell is a "good worry." Try to find the place where your worries will be mostly good. And figure out fast "What does it take to get the job done?"

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