Science stories: flies, planes, worms, and lasers

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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.

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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.

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Abbreviations used: CCD, charge-coupled device; GFP, green fluorescent protein; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

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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