The long road: peering into live cells

Jennifer Lippincott-Schwartz

It was as a high-school teacher in Africa that I realized biology was my destiny. Science teachers were a rare commodity at the rural school in Kenya where I volunteered. There were no textbooks, so I simplified concepts to be copied into students' notebooks, and devised experiences like an overnight field trip to observe wild baboons. The best students hoped for admission to professional school, the only escape from an otherwise subsistence farming lifestyle. On returning to the US, I taught high-school sciences and maths at an elite private school for another two years, in the Stanford area. These privileged students were top calibre, so I had to cram every night just to keep ahead. We made gunpowder, went on outings to the local planetarium and to the mountains to study geology, and impersonated the great physicists of the past century. But I longed to pursue science, not just talk about it.

So I enrolled in the masters programme in biology at Stanford University where now I was the ignorant student in awe of the scientists around me: Arthur Kornberg, Paul Berg and the newly recruited James Rothman, among others. It was like a biology Cannes Film festival.

When I began a Ph.D at Johns Hopkins university, my exposure to molecular biology at Stanford (in particular working in Phil Hanawalt's lab on DNA-repair mechanisms) initially directed me towards work related to DNA replication. However, I soon became aware of the newly emerging field of fluorescence microscopy, where one could watch how cells behaved, not just infer behaviour from biochemical-reaction cascades. This appealed to my artistic nature, so I joined the lab of Douglas Fambrough, who was attaching fluorescent dyes to monoclonal antibodies for visualization of specific proteins within cells.

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Fambrough opened his freezer and said, "Pick any monoclonal antibody you want and study where it targets". I chose a monoclonal antibody that labelled unknown large punctate structures throughout the cell. After co-localizing the structures with acridine orange and performing electron microscopy, I was convinced they were lysosomes and that my monoclonal antibody targeted to a major membrane protein on their surface. But the lysosomal membrane protein seemed to move around in the cell and I could cause it to redistribute to the plasma membrane or endosomes by chloroquine treatment. This is when I came to believe that organelle constituents are dynamic and that to understand organelle biogenesis and structure required knowledge of the intracellular pathways followed by the constituents of the organelle.

As a postdoctoral fellow with Richard Klausner at the National Institutes of Health (NIH), I continued this focus, initially with biochemical pulse-chase labelling, the popular technique of the day. The turning point came, however, when I found that treating cells with the drug brefeldin A led to glycosylation of proteins in the endoplasmic reticulum by Golgi enzymes. At the time, secretory organelles like the Golgi were thought to be stable structures. However, when I used fluorescence microscopy to watch what happened to constituents of the endoplasmic reticulum and Golgi during brefeldin A treatment, the Golgi disappeared, quickly redistributing its constituents into the endoplasmic reticulum. Seeing one organelle disperse into another was an amazing experience.

As a tenure-track scientist at NIH, I decided to use fluorescence microscopy as a primary tool to understand the dynamics underlying organelle structure. Initially, cells had to be fixed and permeabilized to label them, which limits the interpretation of an experiment. Moreover, the dyes used to illuminate the cell, like BODIPY and fluorescein, faded quickly. Then, the first description of green fluorescent protein (GFP) as an exogenous label was published. I jumped at the opportunity to tag our organelle markers with the longer-lasting GFP.

To track proteins with GFP required making movies but I only had wide-field fluorescent microscopes with no camera attachment. So I found a colleague at NIH, Mark Terasaki, who had a confocal microscope and disk drive for collecting and playing back images. Together, we began collecting movies of GFP-tagged membrane proteins. One memorable moment was when we tested whether GFP could be photobleached, as it had been rumoured to be very photostable. We optically zoomed into our GFP-expressing sample to increase the light exposure for bleaching. The sample area went black, as we had hoped. What happened next was unforgettable; the molecules soon began returning into the bleached region showing that their localization in the cell was a dynamic process.

I spent the next few years developing ways to quantify this dynamism using both time-lapse imaging and photobleaching to measure protein diffusion and transport mechanisms. This led to successful efforts to make GFP into a photoswitchable probe, and more recently a tool in super-resolution imaging. Throughout, I collaborated with many exceptional scientists, including biophysicists George Patterson, Michael Edidin and Robert Phair, and physicists Eric Siggia, Eric Betzig and Harald Hess. I also have been very fortunate in having outstanding students and postdocs in my lab, and by being amply supported by intramural NIH funding for cutting-edge microscopes. So, although my pathway to cell biology was never straightforward, it has been a wonderful journey and, I hope, a journey with many secrets of nature still to be revealed working with such talented colleagues.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.