The evolution of a cell biologist

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ABSTRACT **I am honored and humbled to receive the E. B. Wilson Medal and happy to share some reflections on my journey as a cell biologist. It took me a while to realize that my interest in biology would center on how cells are spatially and dynamically organized. From an initial fascination with cellular structures I came to appreciate that cells exhibit dynamism across all scales—from their molecules, to molecular complexes, to organelles. Uncovering the principles of this dynamism, including new ways to observe and quantify it, has been the guiding star of my work.**

INTRODUCTION

That a cell's dynamism is intertwined with cell structure and function, while widely accepted today, was less appreciated when I began my career as a PhD student in the late 1970s. With few time-lapse imaging microscopes, most researchers assumed that cells were composed of static subcompartments intercommunicating via freely floating vesicles or proteins; any dynamism was statistical noise. This fitted nicely with the technologies in use, such as subcellular fractionation to isolate cell components in test tubes, and electron microscopy of fixed specimens.

My earliest interest in cell dynamism dates back to high school. Not thrilled with dissecting frogs and memorizing parts of plants in biology, I remember perking up when my teacher described the mitochondria's role in supplying the cell with ATP. The cell makes and uses energy, I thought—how and why is that happening? Suddenly the cell became an intriguing system that extracted resources from its environment to power cellular functions.

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Upon entering Swarthmore college, my initial goal was to focus on biology, but I eventually majored in philosophy and psychology to explore thinkers who searched for principles underlying science itself. It is humbling to consider that Parmenides and Heraclitus debated more than 2000 years ago whether reality is static or in constant flux, when cell biologists still disagree on what's the more fundamental attribute of cells—their structure or their dynamism.

I next became a high-school teacher in Africa, where I realized that biology was my destiny. Science teachers were rare at the rural school in Kenya where I volunteered. With no textbooks, I had to simplify concepts to be copied into students' notebooks and devised experiences like an overnight trip to observe baboons. My greatest satisfaction was introducing students to the wonders of biology. On returning to the US, I taught high-school sciences and math at an elite private school for another two years, in the Stanford area. I had to cram every night just to keep ahead but it was fun. 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. Still, I longed to pursue science, not just talk about it.

FINDING CELL BIOLOGY

The opportunity arose when I was accepted into the Biology Master's program at Stanford. A molecular biology revolution was unfolding, and many of its superstars were right in front of me: Arthur Kornberg, Paul Berg, and James Rothman, among others. It was awe inspiring to learn from these giants, and I am thankful for the opportunity.

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Abbreviations used: BFA, brefeldin A; ER, endoplasmic reticulum; GFP, green fluorescent protein; PALM, photoactivated localization microscopy.

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When I began a PhD program at Johns Hopkins, my previous exposure to molecular biology, in particular working in Phil Hanawalt's lab on DNA-repair mechanisms, initially directed me to DNA replication work. However, I soon learned of the newly emerging technique of fluorescence microscopy, where cellular behavior could be observed and not just inferred from biochemical reaction cascades. This appealed to my interest in the dynamism within cells, so I joined the lab of Douglas Fambrough at the Carnegie Institute of Embryology. Doug was attaching fluorescent dyes to monoclonal antibodies to visualize specific proteins. Doug opened his freezer one day and said, "pick any monoclonal antibody you want and study where it targets." My choice labeled unknown large punctate structures that I colocalized with acridine orange, a dye known to accumulate in lysosomes. Together with electron microscopy results, I became convinced that my antibody targeted a major membrane protein on the surface of lysosomes. The protein became known as LAMP1. I found it was not stably associated with the lysosome because I could cause it to redistribute to the plasma membrane or endosomes by chloroquine treatment (Lippincott-Schwartz and Fambrough, 1987). This is when I came to believe that an organelle's constituents are dynamic, so understanding an organelle's identity and function would require knowledge of the constituents' intracellular trafficking pathways.

As a postdoctoral fellow with Richard Klausner at the National Institutes of Health (NIH), I continued to focus on organelle trafficking pathways, initially with biochemical pulse—chase labeling. The watershed moment came when I found that treating cells with the drug brefeldin A (BFA) led to mature glycosylation of proteins in the endoplasmic reticulum (ER) by Golgi enzymes. And when I used fluorescence microscopy to watch what happened to Golgi proteins during BFA treatment, the proteins returned to the ER and the Golgi vanished (Lippincott-Schwartz *et al.*, 1989). Seeing one organelle disappear into another was a shock, both to me and to others in the field (including George Palade at Rockefeller University, who soon contacted us to discuss the results). I was then able to show, just as surprisingly, that the Golgi readily reforms upon BFA washout and that this is dependent on ER export activities (Lippincott-Schwartz *et al.*, 1990; Klausner *et al.*, 1992; Sciaky *et al.*, 1997). The experience convinced me that doing science is not so much about dealing with the routine but rather acquiring the skills and temperament to manage biology's surprises. Indeed, this is so vital a part of science training that I believe that programs should push students outside their comfort zones.

DISCOVERIES WITH GFP

As a tenure-track scientist at NIH, I decided to use fluorescence microscopy as a primary tool to understand the mechanisms underlying organelle dynamics, particularly how membrane vesicles navigated through the cytoplasm to different destinations. At the time, observing the process directly was challenging as cells had to be fixed and permeabilized to label them, which did not allow events to be followed in a single cell but rather inferred from different cells fixed at different time points. This dramatically changed with the publication of green fluorescent protein (GFP) as an exogenous label by Marty Chalfie (Chalfie *et al.*, 1994). Now we could attach GFP to our organelle markers and follow organelle behavior and transport carriers in a single cell. My PhD student Nelson Cole obtained the clone, and I remember when he called me to the microscope after he first expressed a GFP-tagged Golgi marker. It was a memorable moment; the cells were alive, and the Golgi shed off long tubules that detached and moved to the cell periphery. This had never been seen before, and I immediately knew that we needed to record and analyze the process.

This required making movies, but we had only wide-field fluorescence microscopes with no time-lapse camera attachment. Fortunately, a colleague at NIH, Mark Terasaki, had a confocal microscope and disk drive for collecting and playing back images. Together, we began collecting movies of GFP-tagged membrane proteins associated with the ER and Golgi. This period was one of the most exhilarating in my career. I'd prepare samples during the day, and we'd spend the evening making movies. Realizing that we were watching events that no one had seen before kept us going late into the night, including in discussions about what we were seeing. One extraordinary time was when we tested whether GFP could be photobleached (Cole *et al.*, 1996). When we focused the full power of our laser line onto our GFP-expressing sample, the sample area went black, but what happened next was even more remarkable: the molecules soon began returning into the bleached region. The molecules were diffusing in a dynamic process! Realizing that we needed to analyze this diffusion quantitatively to make sense of it, I reached out to my Johns Hopkins professor, Michael Edidin (a pioneer in spot photobleaching with fluorescent dyes), and Rockefeller soft-matter physicist Eric Siggia. With their help, as well as input from several talented lab members (including Erik Snapp and later Anne Kenworthy), we spent the next few years developing ways to quantify protein diffusion rates in different environments within cells (Nehls *et al.*, 2000; Kenworthy *et al.*, 2004) as well as tracking the behavior of different GFP-tagged membrane proteins (Ellenberg *et al.*, 1997; Presley *et al.*, 1997; Hirschberg *et al.*, 1998; Zaal *et al.*, 1999; Altan-Bonnet *et al.*, 2003; Polishchuk *et al.*, 2004).

TIME OFF AT THE CAPE

During this period, I had the opportunity to teach at the Marine Biological Laboratory (MBL)'s summer course in physiology at Woods Hole. This experience had a profound impact on my science then and in later years when I came back to join Rob Phillips and Wallace Marshall as codirectors of the course. MBL's mix of energetic students and top-tier researchers not only proved how fun and interactive science can be but helped shape my understanding of the physicochemical properties of cells and their role in driving cell dynamics. Interacting with pioneers like Tim Mitchison, Ron Vale, Julie Theriot, Ted Salmon, Clare Waterman, and others, I learned that cellular structures like actin and microtubules are self-organizing/self-regulating systems existing far from equilibrium. This made me wonder whether my favorite organelle, the Golgi complex, had similar attributes, possibly explaining its unusual capacity for disassembly and reassembly. At MBL, I also saw how important it is to seek out people who challenge your thinking and to listen to uncommon ideas, which is often necessary for real breakthroughs. This is one reason why throughout my career I have regularly attended American Society for Cell Biology meetings and served in leadership positions in the society.

FROM PHOTOBLEACHING TO PHOTOACTIVATION TO SUPERRESOLUTION

Back at NIH, we continued to use GFP to examine the dynamic behavior of organelles, expanding our projects to include mitochondria, lipid droplets, peroxisomes, primary cilia, lysosomes, the ER, the Golgi, and autophagosomes (Kim *et al.*, 2006, 2009; Mitra *et al.*, 2009; Hailey *et al.*, 2010; Rambold *et al.*, 2015; Valm *et al.*, 2017; Figure 1). Lab meetings were always a blast; each postdoc started their presentation by claiming that their organelle was the most exciting. We discovered that we could use photobleaching to measure rate constants for protein movement on/off organelles

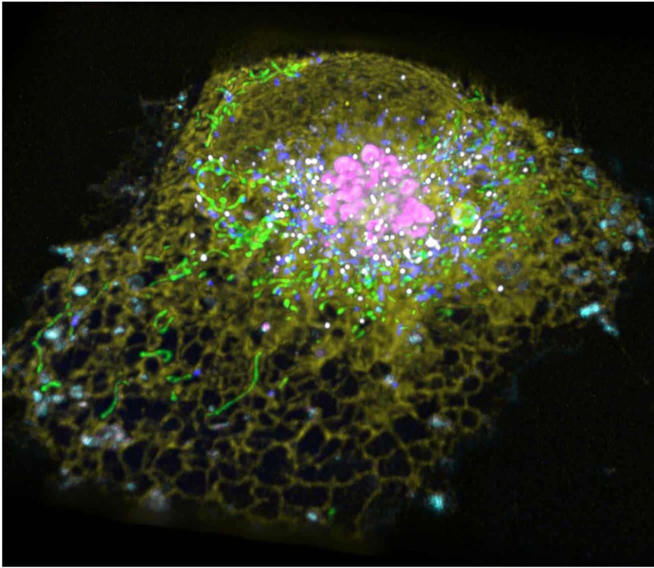

FIGURE 1: The cell's palette of membrane-bound organelles imaged by lattice light sheet multispectral imaging. Organelles are color coded as follows: mitochondria, green; ER, yellow; peroxisomes, blue; Golgi, magenta; lipid droplets, white; and lysosomes, cyan. See Valm *et al.* (2017) for details.

(Presley *et al.*, 2002; Liu *et al.*, 2005) and along membrane trafficking pathways (Nichols *et al.*, 2001; Ward *et al.*, 2001; Patterson *et al.*, 2008). Hoping to improve on this technique, my postdoc George Patterson generated a photoactivatable GFP that could be switched on in time and space. He found that a single point mutation near GFP's fluorochrome could convert GFP to a dark state until switched on by UV light. We called the construct photoactivatable GFP (PA-GFP), and it opened up important new avenues for studying protein dynamics (Patterson and Lippincott-Schwartz, 2002).

The lab was happily using these constructs when I attended a talk by an unemployed engineer from Bell Labs named Eric Betzig. Eric had been thinking intensely about PA-GFP because he believed that it might be the perfect tool to try an approach that he had proposed to break the diffraction limit of light—sequentially turning on individual fluorescent molecules to localize their centers and accumulate their coordinates to create a superresolution image (Betzig, 1995). At lunch with Eric, he laid out the idea that was to become photoactivatable localization microscopy (PALM) for which he received the Nobel Prize. It was elegant and simple. All Eric needed was somewhere to build a microscope and biological hands to prepare probes and handle samples. Soon we were making plans together. Mike Davidson from Florida State University provided additional probes, and our darkroom was outfitted with a home-built microscope based on a prototype by Harald Hess (Eric's close friend from Bell Labs), who also joined our team. Before long, individual photoconvertible molecules were localized at high density on coated-bead aggregates, but identifying structures was more difficult and required extensive troubleshooting of fixation conditions and sample preparation. With George Patterson preparing samples and Eric and Harald managing the scope

FIGURE 2: Current members of my Janelia Lab. Left to right: Ya-Cheng Liao, Andy Moore, Arnold Seo, Chris Obara, Kerry Sobieski (Lab Coordinator), Heejun Choi, Lorena Benedetti, JLS, Peter Rickgauer (Visitor) and Carolyn Ott. Not shown: Chi-Lun Chang, Prabs Sengupta, Dvir Guv and Dani Cai.

through the night to collect as many molecules as possible, a dotted outline of the lysosomal membrane was finally revealed. It was a joyous moment, proving that PALM worked, even inside cells! But were we really looking at single proteins on lysosomal membranes? After much debate, we decided to correlate our images with electron microscopy. Rachid Sougrat, a superb microscopist in my lab, proved up to this difficult task, transferring a viable thin specimen between two microscope systems and relocalizing the sample. When he succeeded, the two images beautifully overlapped with one another (Betzig *et al.*, 2006).

The creation of PALM-based superresolution imaging was possible only through a combination of visionary ideas from physicists, superb configuration of instruments by engineers, unique chemical modifications by clever chemists, and implementation by ever-curious biologists. Soon, my lab began probing the capabilities of PALM for single-molecule tracking and for counting and analyzing molecular distributions (Manley *et al.*, 2008; Sengupta *et al.*, 2011; Renz *et al.*, 2012; Van Engelenburg *et al.*, 2014; Patterson *et al.*, 2010).

A NEW LIFE ON A FARM

Harald and Eric both landed jobs at the Howard Hughes Medical Institute (HHMI)'s Janelia Research Campus to continue their imaging technology innovations. Several years later, I joined them, bringing most of my lab from the NIH. My lab has branched out into several new areas (Figure 2). We are studying sites of mRNA translation, viral budding, interorganelle contacts, phase condensate dynamics, and cell–cell fusion, while maintaining an interest in classic organelles like ER, Golgi, mitochondria, and lysosomes (Nixon-Abell *et al.*, 2016; Seo *et al.*, 2017; Valm *et al.*, 2017; Cai *et al.*, 2019; Chang *et al.*, 2019; King *et al.*, 2019; Liao *et al.*, 2019; Sengupta *et al.*, 2019). Janelia's unique combination of four-dimensional imaging capabilities, chemical probe development, protein-based sensor engineering, and innovative microscopes has proven to be an ideal environment. The theme of cellular organization and dynamism still remains my lab's focus, as it has throughout my career. But now we are looking at it from a broader perspective, including in complex environments such as tissues.

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I thank all my collaborators and terrific graduate students, postdocs, and research fellows over the past 30 years, whose amazing ideas and experimental prowess has made it possible to carry out our journey into the interior of cells. We have had many fun and exciting times together. For my current research, I especially thank my senior staff scientists, Carolyn Ott, Prabs Sengupta, and Chris Obara, for their unstinting contributions and support to our scientific family, and I am grateful to the rest of my postdocs, Arnold Seo, Heejun Choi, Chi-Lun Chang, Ya-Cheng Liao, Dvir Guv, Dani Cai, and Andy Moore, for their spirit and forbearance during this challenging period. Finally, I thank the NIH and HHMI for generously supporting my work through the years.

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