

Life in a Three-dimensional Grid

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There have been two sharp demarcations in my life in science: the transition from fine arts to chemistry, which happened early in my career, and the move from New York to Stanford University, which initiated an ongoing collaboration with the physicist Harley McAdams. Both had a profound effect on the kinds of questions I posed and the means I used to arrive at answers. The outcome of these experiences, together with the extraordinary scientists I came to know along the way, was and is an abiding passion to fully understand a simple cell in all its complexity and beauty.

The New York Subway System

I was born in Brooklyn, the eldest of three daughters. My mother, an elementary school teacher, and my father, a Ukrainian immigrant, shared an unwavering belief that it was their duty to surround me with all the riches of a multifaceted education. At age 13, I set my sights on the High School of Music and Art as a way of avoiding attendance at my neighborhood Manual Training High School. As I hauled my portfolio of drawings and paintings from the depths of Brooklyn to West 137th street in Manhattan for the Music and Art entrance exam, I had my first taste of the two-hour subway stint (each way) that was to be my lot for the next four years. Life in the subway included homework, reading musical scores, and filling sketch pads with portraits of my co-travelers. Although my high school curriculum was overwhelmingly geared to the arts, an inspiring biology teacher, Mr. Mirelman, introduced us to the beauty of the living world, an introduction that turned out to have deep roots. After a wonderful experience at the High School of Music and Art, I attended another New York institution, Brooklyn College. My subway trajectory altered, but I remained in the subterranean travel world. I was selected for the honors program, in which I had freedom to shape my own education, so I sampled many disciplines, including a major in biology, while also focusing on my training in fine arts and art history. I was fascinated by the Italian Renaissance, so I chose Dante Alighieri and the forces that drove him to compose the *Divine Comedy* in the vernacular rather than Latin as the subject of my senior paper. Just after graduating from college, I experienced one of those critical moments that change the course of one's life. I was introduced to Ted Shedlovsky, a physical chemist at Rockefeller University. Shedlovsky was known for, among many other accomplishments, his sponsorship of the Friday concert series at Rockefeller University. He had a history of encouraging students in the arts to delve deeply into science. At his instigation, I took an honors course in organic chemistry, although I had none of the prerequisites. This was a transformative experience, and I fell in love with the clarity and elegance of the chemical world. With Shedlovsky as a mentor, within a year, I was matriculated in the Ph.D. program at the New York University (NYU) School of Medicine in Bernard Horecker's Department of Microbiology. Under the guidance of Tom August (my Ph.D. advisor) and Jerry Hurwitz (an exemplary and ongoing scientific mentor to this day), a plan was put in place for me to acquire the analytical underpinnings I would need for graduate work in biochemistry (Fig. 1).

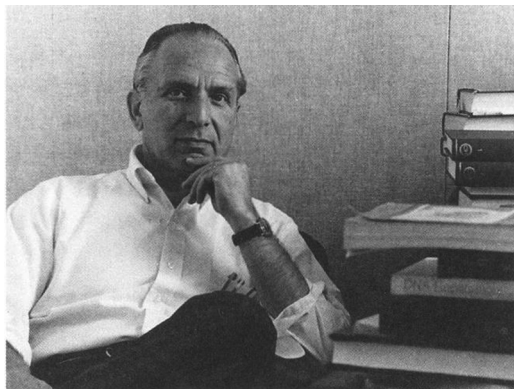


FIGURE 1. Jerry Hurwitz (2006) at the Memorial Sloan-Kettering Cancer Center.

Again, I was crisscrossing the city in the subway at all hours of the day and night to take courses in physics, physical chemistry, biochemistry, and even molecular orbital theory at the downtown campus of NYU, the NYU School of Medicine, and Columbia University. Hurwitz made it clear that anything less than an “A” in any course would mean the end of my graduate career. I believed him! A highlight of this period was having Severo Ochoa, Charles Gilvarg, and Peter Lengyel as my teachers in the memorable NYU School of Medicine biochemistry course. I remember well Ochoa’s enthusiasm for biochemistry and his pronunciation of “*fructose*” 6-phosphate when discussing intermediary metabolism. At that time, we still had to crawl around in dusty library stacks to explore the literature instead of our current access on the Internet. It was in the NYU School of Medicine library stacks, trying to solve innumerable problem sets, where I spent many an inspirational hour with Gilvarg discussing biochemistry. Gilvarg introduced me to Ernest Baldwin’s *Dynamic Aspects of Biochemistry*, which I continue to consult to this day. Although immersion in the Internet is a rich and rewarding enterprise, it is displacing one-on-one interactions with live teachers and mentors that for me were an essential aspect of becoming a scientist in my own right.

RNA Polymerase

By 1963, the Hurwitz and August laboratories (first at NYU and then at the Albert Einstein College of Medicine after Horecker’s entire department moved as the new Department of Molecular Biology), were the hub of RNA polymerase enzymology. The classic 1961 *Cold Spring Harbor Symposia on Quantitative Biology* volume included François Jacob and Jacques Monod’s seminal paper describing the mRNA hypothesis and the proposed function of *trans*-acting transcriptional regulators (1) and Hurwitz’s paper on *Escherichia coli* RNA polymerase

defining the enzymatic mechanism of DNA-directed RNA synthesis (2). The ensuing flow through our laboratory of visitors who wanted to learn, hands on, the biochemistry of these enzymes included Monod and David Baltimore. This created an intellectual foment that was heady medicine for the young graduate student I was then. Late-night sessions with Hurwitz, August, the visitors, postdoctoral fellows, and students included Stan Cohen, Ann Skalka, Carol Prives, and Malcolm Gefter. These sessions were challenging and exciting, but most importantly, they taught me the transient nature of proposed mechanisms and constructs that were passionately promoted and discarded. Hurwitz was a remarkable teacher and biochemist who instilled in us a passion for critical thinking and accuracy and the love of a beautiful experiment. The report by Norton Zinder in 1963 of phage $\phi 2$, the first RNA-containing bacteriophage, initiated a flurry of activity to identify an enzyme that replicated single-stranded RNA. Obtaining the $\phi 2$ phage from Zinder proved to be part of the challenge. My first publication, with Tom August and Zinder as senior author, reported RNA-dependent RNA polymerase activity in phage $\phi 2$ -infected *E. coli* (3). This paper followed closely upon a report from the NYU Department of Biochemistry, a floor above us, by Charles Weissmann, Lionel Simon, and Ochoa of an RNA-dependent RNA polymerase from phage MS2-infected *E. coli* (4) and one from Baltimore and Richard M. Franklin using mengovirus-infected cells (5). During my graduate work on the RNA phage polymerase, I got to know and argue with Sol Spiegelman, eventually presenting a talk at a meeting describing data that differed from his current model of phage Q β replication while he stood in the back of the auditorium kindly holding Peter, my infant son, who was born just after I finished my graduate work.

As I was writing my thesis, Dick Bellamy, working just upstairs in Bill Joklik’s laboratory, was wrestling with reovirus RNA. Together, Bellamy and I tried to understand the aberrant behavior of this viral RNA. Using physical chemical analyses and the old space-filling models (which filled up the living room in my small apartment), we demonstrated that the reovirus genome existed in multiple segments of double-stranded RNA (6). (While growing the vast amount of reovirus we needed for our experiments, both Bellamy and I came down with serious reovirus respiratory infections.)

The Bacterial Cell as a Three-dimensional Chemical Machine

With a firm grounding in biochemistry and plans in 1966 to do postdoctoral work on the hydrogen bond with



FIGURE 2. Lucy Shapiro (1972) at the Albert Einstein College of Medicine.

Walter Kauzmann at Princeton University, I was set adrift when Kauzmann had to take an unplanned leave of absence. I spent several months with Julius Marmur at the Albert Einstein College of Medicine as a Jane Coffin Childs Fund Fellow. Horecker then asked me if I would join his Department of Molecular Biology at Einstein as an assistant professor, but, if I should agree, he advised that I take three months to stop and think about the focus of my research program. I can not imagine that happening today, as postdoctoral fellows now spend multiple years demonstrating that their research programs are quite established and that they can hit the ground running with National Institutes of Health support imminent. For me, this mandated time to think and read outside of my comfort zone was an incredible gift. Throughout all of my training as a biochemist, I had felt constant concern about what our *in vitro* characterized reactions were actually doing in the cell, where Michaelis-Menten kinetics were surely not the norm. Furthermore, the cell and its contents exist in three dimensions. Picking up from the 1961 Jacob and Monod *Cold Spring Harbor* paper, it was clear to me then that the cell regulatory mechanisms and enzymatic pathways that controlled a living cell had to function as an integrated network with my added proviso that they must operate in the cell in a three-dimensional framework. I set out to search for the simplest cell that exhibited polarity and asymmetric cell division so that I could manipulate the spatial dimension in an attempt to study the *in vivo* biochemistry of regulatory pathways (Fig. 2). Although my knowledge of microbiology at the time was rudimentary, after extensive reading of the microbiology literature, I focused on the stalked bacterium *Caulobacter crescentus*, which exhibited a dimorphic cell cycle. Most importantly, prior to cell division, one end of the cell sported a stalk and the other a flagellum, so there were observable and distinct polar markers. Each cell division yielded progeny that differed morphologically and functionally, which we now recognize as a basic paradigm of stem cell asymmetry.

Here was my “simple” cell! No matter that it could then barely be grown in defined media, that there was no genetics to speak of, and that understanding its biochemistry was a distant dream. When I informed Hurwitz of my plan to focus on *Caulobacter*, he was aghast. “How can you waste all the clean chemistry we taught you on such *drek*?”

Roger Stanier and his students Jeanne Poindexter and Jean Schmidt established the basic microbiology of *Caulobacter*, and they showed that it was possible to obtain synchronized cell populations, which has proved to be an invaluable procedure for our studies of the genetics and biochemistry of cell cycle progression. Stanier believed that *Caulobacter* is a valuable organism in which to study cell differentiation because programmed changes in cell morphology are integral to cell cycle progression. At the onset of my life as an independent scientist, I mapped out an approach to ultimately understand how this relatively simple Gram-negative bacterium functions as an integrated system operating in both time and space. The strategy was to proceed in pyramid fashion, pursuing specific milestones: 1) identify polar organelles and generate antibodies so that sequential changes in polar morphology could be followed during an individual cell cycle and 2) sort the cell cycle into workable modules and define the biochemistry underpinning each module. These modules include chromosome replication; the biosynthesis and positioning of the cell division apparatus; flagellum biogenesis; synthesis of the chemotaxis machinery; DNA methylation; and chromosome architecture and segregation. Concomitantly, new imaging technologies would be needed to understand the cell biology of these tiny cells to integrate the regulation of the expression of each module with the dynamic nature of cell structure leading to asymmetric cell division.

Initially, a paramount objective was the establishment of *Caulobacter* genetics. Then at an early point in his career, Bert Ely, a graduate student at The Johns Hopkins University with Phil Hartman, visited my laboratory and stated quite simply that *Caulobacter* needed a genetic system and that he, with then-high-school-student Reid Johnson, would do just that. Ely, as a new assistant professor at the University of South Carolina, and Johnson isolated hundreds of mutants and, using a transduction system, generated the first full genetic map of *C. crescentus*.

A major shift in our approach to the integration of the cell cycle regulatory pathways that drive the *Caulobacter* cell cycle occurred upon my move (after having already moved from Einstein to the Columbia College of Physicians and Surgeons to serve as Chair of the Department of Microbiology) to the Stanford University School of Med-

icine in 1989 to establish the new Department of Developmental Biology. Three events dramatically changed the trajectory of our work: our demonstration of dynamically localized signaling proteins in bacterial cells, the establishment of an interdisciplinary laboratory based on a collaboration with the physicist Harley McAdams, and the discovery that a remarkably small number of transcription factors control the sequential activation of all the modules that accomplish the *Caulobacter* cell cycle. We soon identified the first coherent and integrated genetic circuit model for cell cycle control.

Demonstration of Dynamic Subcellular Localization of Signaling Proteins

In the 1980s, it was commonly held that bacteria are essentially bags of enzymes with a floating nucleoid and that any molecule could get to any place in the cell in milliseconds by free diffusion. Janine Maddock and Dickon Alley, early postdoctoral fellows in my new Stanford laboratory, established first in *Caulobacter* (7) and then in *E. coli* (8) that the chemoreceptors involved in chemotaxis are located at the pole of the cell. Maddock's innovative experiments using immunogold electron microscopy on thin sections of *E. coli* as well as indirect immunofluorescence on these 2- μm cells got the attention that eluded comparable experiments that we had previously reported on *Caulobacter* cells. There was a lag of a few years before the chemotaxis community adopted the paradigm of subcellular protein localization into its mechanistic models of chemotaxis.

Several years later, Christine Jacobs, as a postdoctoral fellow in the laboratory, and Maddock demonstrated that an essential histidine kinase localizes to the cell pole at a specific time in the cell cycle where it mediates a phospho-signaling cascade that controls cell cycle progression (9). To do these experiments, Jacobs traveled to Rich Losick's laboratory at Harvard University to obtain time-lapse images of fluorescently tagged CckA, mostly working at night when the microscope was available. The three-hour lag allowed us to share our excitement in real time as the data rolled in. (Jacobs, now Jacobs-Wagner, is a professor at Yale University and is continuing the exploration of *Caulobacter* cell biology.) By 2006, we had discovered that the ClpXP protease, in addition to signaling and chemotaxis proteins, was dynamically localized to and functioned at specific subcellular locations (10), which proved to be the case in every other bacterium studied (11).



FIGURE 3. Harley McAdams and Lucy Shapiro (2009) at the John Scott Award Ceremony.

Establishment of an Interdisciplinary Laboratory

My husband, Harley McAdams, is a physicist who left Bell Laboratories in 1989 to move across the country with me to Palo Alto. After our move, he initially worked in the aerospace industry, but he made a major career change in 1994 when he concluded (after listening to my constant talk about *Caulobacter*) that the cell cycle control system must have a close analogy to the control systems he was familiar with in electrical systems. He soon resigned from the Lockheed Missile and Space Company and set out to demonstrate this using the well studied regulation of phage λ infection of *E. coli* as a paradigm (12). Harley's foray into becoming bilingual in physics and genetics resulted in a major shift in our work on the *Caulobacter* cell, which we now saw, even more than previously, as an integrated system. In the Department of Developmental Biology at Stanford, where Harley became a professor, his students were largely physics and electrical engineering graduate students who joined his laboratory because of their interest in biological questions. In a pioneering approach for the time, we integrated our laboratories to create an interdisciplinary group that was able to combine sophisticated imaging algorithms, biocomputation, and simulations of the genetic circuitry with biochemistry and molecular genetics. In 2009, we were the first couple to be presented with the John Scott Award in the ~ 170 years since its inception (Fig. 3). Together, we have shown that the control circuitry that directs and paces the cell cycle involves the entire cell operating as an integrated system and that bacterial cell cycle control is exquisitely optimized as a total system for robust operation in the face of internal stochastic noise and environmental uncertainty (13, 14).

During this period, full genome sequencing gave us an annotated version of the *Caulobacter* chromosome (15) that has by now been refined many times over by ribosome

profiling,¹ identification of the essential genome at 8-bp resolution (16), and identification of the global transcriptome (17). Mike Laub, as a graduate student in our laboratory in 1999 (and now a professor of biology at the Massachusetts Institute of Technology), co-opted an early microarray apparatus in the basement of the Beckman Center at Stanford between midnight and 4 a.m. for several weeks in 2000 to carry out the first cell cycle microarrays of bacterial transcription patterns. He produced a dramatic demonstration that the bacterial cell cycle is globally organized by the sequential activation and repression of genes (18). He also showed that more than 250 genes are dynamically expressed as needed for each cell cycle functional module. We found that execution of a cell cycle-dependent event correlates with the time of expression of genes required for that event and that genes encoding proteins that function together as molecular complexes are often coexpressed.

Discovery That a Small Number of Regulatory Proteins Control the Cell Cycle and the Establishment of an Integrated Genetic Circuit

Our Stanford laboratory was actively pursuing the components and function of each of the cell cycle modules, including chromosome replication, biogenesis of the divisome, flagellum and pilus synthesis, transient assembly of the polar chemotaxis apparatus, and chromosome methylation and segregation. A critical genetic screen was performed by a graduate student in the laboratory, Kim Quon, that led to his discovery of the CtrA transcription factor as a global regulator of all of these modules (19). CtrA ultimately was shown to directly control the temporal transcription of more than ninety-five genes and operons (20). CtrA, a two-component system-response regulator, was shown to play two roles in cell cycle control: one as a transcription factor and the other as a negative regulator of replication initiation. In its phosphorylated state, CtrA binds to and silences the origin of replication in swarmer cells. Once CtrA is cleared from the cell by ClpXP-mediated proteolysis upon swarmer cell-to-stalked cell transition, the DnaA protein binds to the origin and enables replication initiation. Like CtrA, DnaA is also a dual-function protein, serving both as a positive regulator of replication initiation and as a transcription factor that controls more than forty cell cycle-regulated genes. DnaA turns on the transcription of a gene encoding GcrA, a third global regulator. Together, these three temporally controlled transcription factors form an oscillating circuit, control-

ling cell cycle progression. We made the surprising discovery that the transcriptional circuitry is temporally linked to chromosome replication by virtue of changes in DNA methylation state concomitant with replication fork progression (21, 22). As replication proceeds, the DNA sequentially goes from the fully methylated state to the hemimethylated state. The origin-proximal *dnaA* gene is preferentially transcribed from a fully methylated promoter, whereas the origin-distal *ctrA* gene is transcribed from a hemimethylated promoter. Superimposed on the transcriptional circuitry and functioning to regulate the activity of the CtrA transcription factor is a network of dynamically localized phospho-signaling proteins (23) that drive the cell cycle, establish asymmetry, and create two distinct daughter cells, presenting a strong parallel to the strategy used by eukaryotic cells for cell cycle and stem cell control.

Coda

None of the work I have distilled to its essence in this Reflections article would have been possible without the extraordinary group of students and postdoctoral fellows with whom I have shared my forty years in science, several of whom I have mentioned. They consistently provided novel, challenging, and exciting approaches to the study of the bacterial cell. Twenty of these talented scientists are now running their own highly successful *Caulobacter* laboratories in multiple countries. Now, the next generation is establishing an ever-expanding group of investigators in this field. One of the most meaningful and rewarding aspects of my life has been the opportunity to mentor and support young scientists.

I am indebted to the National Science Foundation, which took a chance on funding a new and very young investigator proposing to explore the improbable idea that the simple bacterial cell operates as an integrated system encompassing time and space. This initial support was followed by a steady stream of funding from the National Institute of General Medical Sciences that has simply made everything possible. Finally, Stanford University and its cadre of truly exceptional and giving scientists have provided very rich soil in which to grow innovative science.

It is not possible to end this brief excursion through my scientific life without acknowledging the friends with whom I shared the excitement and wonder of the discovery process over a lifetime and across continents. These include Charles Babinet from the Pasteur Institute, Ann Skalka from the Fox Chase Cancer Center, Susan Henry from Cornell University, Carol Prives from Columbia Uni-

¹ J. Schrader, J. Weissman, and L. Shapiro, unpublished data.



FIGURE 4. Lucy Shapiro and Rich Losick (1985) at Cold Spring Harbor.

versity, and Rich Losick (Fig. 4) from Harvard. Although we were never at the same institution, none the less the same city, Losick and I have remained close friends and intellectual collaborators for almost our entire lives in science. As young investigators, me at Einstein and Rich at Harvard, we were both scheduled to present talks at the Regulation Gordon Conference in New Hampshire. Because neither of us was working on *E. coli*, our talks were relegated to the Friday morning session, which routinely housed topics that did not fit into a recognized category. On that Thursday night, Don Brown advised us to limit our talks to two or three slides. In our ensuing panic, we decided to rehearse our talks, by then 10:00 at night. Rich was aghast when he saw my complicated slides. He promptly disappeared and then reappeared with a small role of black electrical tape and proceeded to blank out whole sections of data. (Ah, the era before PowerPoint!) From that time on, we shared not only our communication skills but also our dissection of bacterial genetic networks that we showed, in both *Caulobacter* and *Bacillus subtilis*, to operate in a three-dimensional grid.

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