Twists and Turns: My Career Path and Concerns About the Future

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THE Genetics Society of America’s Thomas Hunt Morgan Medal is awarded to an individual GSA member for lifetime achievement in the field of genetics. The 2014 recipient is Frederick Ausubel, whose 40-year career has centered on host–microbe interactions and host innate immunity. He is widely recognized as a key scientist responsible for establishing the modern postrecombinant DNA field of host–microbe interactions using simple nonvertebrate hosts. He has used genetic approaches to conduct pioneering work that spawned six related areas of research: the evolution and regulation of Rhizobium genes involved in symbiotic nitrogen fixation; the regulation of Rhizobium genes by two-component regulatory systems involving histidine kinases; the establishment of Arabidopsis thaliana as a worldwide model system; the identification of a large family of plant disease resistance genes; the identification of so-called multi-host bacterial pathogens; and the demonstration that Caenorhabditis elegans has an evolutionarily conserved innate immune system that shares features of both plant and mammalian immunity.

I was born on VJ day, September 2, 1945, the official end of World War II and the first day of the baby boom generation. It was an auspicious time for a future scientist to be born. I entered the job market in 1975 when interest in and funding of science was expanding and when academic jobs were relatively plentiful.

The path I have taken from being a college chemistry major to a molecular biologist and geneticist has had many twists and turns. Chance encounters and unplanned events played an important role in shaping my career. The political and social upheavals of the 1960s also greatly influenced my career choices, as did a psychological restlessness that made it difficult for me to focus for any extended period on a particular project or goal. Although I would not necessarily recommend that anyone follow my career path, I suspect it would be a much more treacherous journey today than it was 50 years ago, reflecting what appears to me to be a degradation in the ethos of the scientific community.

How the First Premier of China Inspired Me to Become a Plant Biologist

As an undergraduate chemistry major at the University of Illinois in Urbana-Champaign, I did not have a clear idea of what I was going to do after college until I attended a standing-room-only seminar by Sol Spiegelman on his demonstration of phage Qβ replication in vitro. I decided then that I wanted to become a molecular biologist. It was not a difficult decision as a new graduate student at the Massachusetts Institute of Technology (MIT) to join Ethan Signer’s laboratory in 1966. Ethan himself had joined the MIT faculty that same year after his postdoctoral training with Sydney Brenner at the MRC lab in Cambridge England, and Francois Jacob and Jacques Monod at the Institut Pasteur in Paris.

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—F.M.A.

My first project as a first-year graduate student was to obtain evidence, lacking at that time, that lac repressor binds to DNA. Ethan had calculated that if repressor was bound to the DNA of a λ lac-transducing phage and was packaged into the phage head, it would change the density of the phage just enough to be detectable on a cesium chloride gradient. The experiment was elegant in its simplicity. 14C-labeled lac-transducing phage grown in the absence of isopropyl β-D-1-thiogalactopyranoside (IPTG) mixed with 3H-labeled phage grown with IPTG and vice versa. (IPTG is a stable synthetic lactose mimic that binds lac repressor, causing it to dissociate...
from DNA.) In the same gradient, $^{14}$C- and $^3$H-labeled wildtype λ, which had a distinct density from the lac-transducing phage, served as an internal control in the gradient. Unfortunately, I never detected a density difference between the $^3$H- and $^{14}$C-labeled λ lac-transducing phages, presumably because the repressor was stripped from the DNA during packaging. Nevertheless, it was a marvelous introduction to the thinking behind and practice of molecular biology and illustrated to me in a very powerful way that relatively simple experiments can yield important new insights.

My graduate student years, 1966–1972, were notable not only because of the seminal discoveries being made in molecular biology, but also because of the political and social upheavals occurring as a consequence of the Vietnam war and the bombing of Cambodia. It felt as if I had two full-time jobs, working on my PhD thesis on the one hand and being a political operative on the other. I was newsletter editor for the major graduate student antiwar organization at MIT, the Science Action Coordinating Committee, which regularly lambasted MIT’s involvement in weapons-related research. I was also an active participant in Science for the People, an organization founded on the principle that science, including genetic engineering, should benefit the poor and disenfranchised rather than being used to benefit militarism and the wealthy classes.

In 1971 and 1972 when I was finishing my PhD work on λ integrase, prominent molecular biologists such as Gunther Stent and Jim Watson were saying that the golden age of molecular biology was over; they advised graduate students and postdocs to stop working on *Escherichia coli* and its phages and to seek opportunities for carrying out work in eukaryotic model systems. Although many of my classmates made the switch with relative ease, it seemed a complicated choice to me. I was indecisive about what to do next.

In 1971, Ethan, my advisor, and Arthur Galston from Yale traveled to Vietnam and China. They were the first American scientists to visit the People’s Republic of China since its founding in 1949, and while they were in China they had the opportunity to meet with Chou En-Lai, the premier of China and the right-hand man of Mao Zedong during the revolution. Ethan came back from this trip enthused about doing “science for the people,” especially agricultural science.

I started thinking about plant-related science as a potential future field, which finally materialized into a decision to work on nitrogen fixation, a microbial process, but important for agricultural yield. I decided to construct a λ-like phage to transfer the nitrogen fixation *(nif)* gene cluster from *Klebsiella pneumoniae* to plant cells in the hope of engineering a nitrogen-fixing plant! While looking into the literature for a suitable plant host, I came across a large body of genetic work on *Arabidopsis thaliana*, which appeared to me to be a potential “Drosophila” of the plant world. Although transferring functioning bacterial *nif* genes to plants seems hopelessly naive in retrospect, in these pre-cloning days it was considered a cutting-edge strategy.

I arranged a 2-year postdoctoral fellowship to work on the *nif* gene project. The plan was to spend the first year at the universities of Leicester and Nottingham to work with *Arabidopsis* tissue culture cells and protoplasts with H. E. Street and Edward Cocking, respectively. In the second year, I would bring the tissue culture and protoplast systems to the lab of Lawrence Bogorad at Harvard and work on the *nif* gene transfer. This plan did not go well. Working with *Arabidopsis* tissue cultures and protoplasts was extraordinarily tedious compared to bacteria and phages, and I became quite discouraged. I was not aware of anyone else who was working to develop *Arabidopsis* as a model plant for molecular genetic analysis, and I could not see a clear career path that involved the use of *Arabidopsis* tissue culture.

Fred is a pioneer. He fathered scientific fields that have now grown to hundreds of different research groups.

—Gary Ruvkun, Harvard University

In a stroke of good luck, however, Stanley Cohen at Stanford and Herb Boyer at University of California at San Francisco invented cloning while I was in England and I found that *K. pneumoniae* could be easily transformed with pSC101, Cohen’s and Boyer’s original cloning vector. I realized that the strains that I had constructed that were deficient in both nitrogen fixation and restriction/modification could be used to clone *nif* genes. In another stroke of luck, I met Frank Cannon from the Unit of Nitrogen Fixation at the University of Sussex, and he invited me to his lab to work on the *nif* gene-cloning project. The switch temporarily ended my work with *Arabidopsis*, and I did not take it up again for almost 10 years.

When I moved to the Bogorad laboratory in 1974, I received a National Science Foundation (NSF) postdoctoral fellowship entitled “Transfer of functioning nitrogen fixation genes to plants.” The plant in the NSF application was *A. thaliana*. In 1975 I became an assistant professor in the Cellular and Developmental Biology Department at Harvard and my NSF fellowship was converted to a grant.

Unexpectedly, I received quite a bit of pushback on the *nif* gene-cloning project from my former colleagues at Science for the People, who wanted me to take a stand against cloning. This confused me because one of the major drivers in my decision to transfer *nif* genes to plants was to benefit developing countries and the environment. In any case, work on the project largely came to standstill in 1975 following the Asilomar Conference on Recombinant DNA and the establishment of guidelines for carrying out recombinant DNA work. I was using *K. pneumoniae*, a human pathogen, as a potential host for recombinant plasmids carrying *nif* genes. This was strictly forbidden in the original guidelines, but recombinant DNA guidelines were eventually relaxed.
after a couple of years, and the first nif genes were cloned in 1977 (Cannon et al. 1977).

In shifting from chemistry to molecular biology to plant biology, I had simply followed my inclinations to carry out work that I perceived to be on the cutting edge scientifically, as well as socially important. When I started on the quest to transfer nif genes to plants, there was no clear path to success, and the entire venture could easily have been considered foolhardy. Nevertheless, I was able to obtain a postdoctoral fellowship (with a little help from Salvador Luria) and forge a somewhat unusual program to pursue my long-term goals. After Cohen and Boyer, the nif gene part of the project suddenly became much more feasible, but the transfer of functioning nif genes to plants was most likely unachievable, given the fundamental differences in the structures of prokaryotic and eukaryotic genes, which became apparent only in subsequent years, and the fact that the K. pneumoniae nif gene cluster consists of 17 genes. Nevertheless, as stated above, I was awarded an NSF grant to pursue the project. Indeed, the NSF proposal was very highly ranked.

I toned down subsequent applications as the complexities of the nitrogen fixation system came into focus. But I doubt whether postdoctoral and NSF applications comparable to the ones that I wrote in the early 1970s would be funded today. I did not have three clearly achievable specific aims that were interrelated but did not depend on each other and that did not involve a genetic screen. The aims were based on minimal preliminary data, and it was not obvious that a complete functioning nif gene cluster could be readily cloned or that bacterial genes could be expressed in plants. The aims were innovative but highly speculative and overambitious.

### Switching Focus, Again

In 1977, there was still lot of work to be done on elucidating how the Klebsiella nif genes were organized and regulated, but I was not content to limit the lab to this complex project. Soon after I started my lab at Harvard, we were working on several distinct projects, including (1) identifying and cloning nif and nodulation-related genes in Rhizobium meliloti, the nitrogen-fixing symbiont of alfalfa; (2) identifying alfalfa genes involved in nodulation; and (3) developing petunia as a model system in which to transfer nif genes. In those days, petunia was much more amenable to tissue culture than Arabidopsis. The petunia work turned out to be a dead end, but we were able to demonstrate that nif genes are highly conserved in evolution (Ruvkun and Ausubel 1980). We successfully developed techniques to mutate specific Rhizobium genes (Ruvkun and Ausubel 1981), thereby allowing us to study the organization and regulation of Rhizobium nif genes (Ruvkun et al. 1982) and to clone Rhizobium nod genes (Long et al. 1982). The study of nif gene regulation resulted in the discovery of two-component regulatory systems, which are widely dispersed in prokaryotes and consist of an environmental sensor protein and a response regulator protein that activates gene expression (Nixon et al. 1986). We initiated work to investigate the regulation of legume genes involved in nodule development and function (Dickstein et al. 1988).

In 1982 I was recruited by Howard Goodman, one of the pioneers of recombinant DNA technology, to the newly formed Department of Genetics at Harvard Medical School and Department of Molecular Biology at Massachusetts General Hospital. At this time I was firmly committed to studying host–microbe interactions but I was disillusioned about working on legumes because of the inability to readily carry out genetic analysis of host genes. In 1983 I heard a lecture by Christopher Somerville at a Gordon Conference on the identification of Arabidopsis photosynthesis-related mutants, leading me to conclude that it was a propitious time to return to Arabidopsis. With the ability to construct physical-genetic maps using recombinant DNA technology, it was now possible to use a chromosomal walking strategy to clone an Arabidopsis gene identified only by its phenotype. However, because Arabidopsis is not a legume, we could not use it to study symbiotic nitrogen fixation. So I gradually switched the focus of the lab from plant symbionts to plant pathogens. Similarly, instead of the plant nodulation response to Rhizobium, we studied the plant innate immune response to pathogen attack. This led to the cloning of the founding member of a large family of plant genes that confer resistance to particular strains of pathogens (Mindrinos et al. 1994) and the use of forward genetic analysis to dissect the components of the plant immune response (Glazebrook et al. 1996). This was the first time that forward genetic analysis had been used to study the process of host immunity in a higher eukaryote.

Laurence Rahme joined the lab in 1992 as a postdoctoral fellow after completing her PhD at the University of California at Berkeley. At Berkeley, she had learned about experiments showing that the opportunistic human pathogen Pseudomonas aeruginosa could also infect plants. However, it was not known whether the same P. aeruginosa strain could infect both plants and animals. She reasoned that P. aeruginosa strains that were infectious in both plants and animals could be used to identify key features of pathogenesis that were independent of the host. It turned out that many P. aeruginosa strains were infectious in Arabidopsis, and Laurence chose for further study one of these strains, UCBPP-PA14 (PA14), which was also highly infectious in mice (Rahme et al. 1995). When Man-Wah Tan, a graduate student, showed that PA14 also killed Caenomorhabditis elegans, we started a C. elegans project designed to identify PA14 virulence factors on the one hand and C. elegans immune genes on the other (Tan et al. 1999). This is now the major project in the lab.

### Concerns About the Future

One of the reasons I have not been able to drill down very often on particular projects to their mechanistic underpinnings
is that I have always let my postdocs take their projects with them. But I also just happen to be somewhat restless and more interested in starting new projects than finishing old ones. This has always served me well, and until recently it was not a problem getting a grant funded that was not an obvious extension of the projects in a previous grant. The former Genetics Study section at the National Institute of General Medical Sciences was content with the lab's record of opening up new areas of research. I did not have to determine the underlying details of the molecular mechanisms because I could explain that former postdocs had taken these projects to start their own laboratories. The Genetics Study section was also content when I proposed to extend the genetic analysis of innate immunity from C. elegans to Arabidopsis, making the argument that the two projects might be synergistic. Nobody questioned whether I was "qualified" to carry out C. elegans research or whether the study of plant and nematode immunity was relevant to human disease, and no one raised a question about the biological relevance of studying immune responses in an artificial laboratory assay significantly different from the ecological niches in which Arabidopsis and C. elegans encounter pathogens in the wild.

Although many of my former postdocs have started their own laboratories based on studies of Arabidopsis or C. elegans innate immunity, it appears that this is becoming more difficult. NSF support for Arabidopsis genetic resources appears to be in doubt. At the National Institutes of Health, concerns are often raised about the relevance of model organisms, given rapid advances in our ability to identify and manipulate human genes correlated with disease. This should be troubling not only to those who study model genetic organisms, but also to everyone in the scientific community committed to the importance of basic science.

There are other obstacles for aspiring young scientists that I did not have to face. Obstacles include not only the very low funding rates, but also the funding priorities that favor the larger "pork barrel" projects over smaller investigator-initiated grants; the exaggerated influence of Science, Nature, and Cell on hiring, promotion, and funding decisions and the corresponding pressure to publish in these journals; the soft academic job market and the precariousness of soft money positions for PhD faculty at many medical schools; the continuously rising bar for publication in mainstream journals, fueled in part by development of powerful new genomic technologies (that are often quite expensive) and the demands of referees for months of additional experiments. As I reach the end of my career, I find these obstacles daunting, even though they affect me only indirectly through the difficulties my students and postdocs encounter as they attempt to thread their way through this career minefield.

What I find particularly unfortunate is that these trends are arising during an unprecedented explosion of biological knowledge as a consequence of "next-generation" genomic techniques. Full-genome sequencing, RNA interference, and CRISPR (clustered regularly interspaced short palindromic repeats) technologies allow almost all biologists to carry out sophisticated genetic analysis and have enabled previously unthinkable evolutionary studies. Genome-wide association studies have revolutionized human genetic analysis, and whole-genome sequencing has created a renaissance in the study of prokaryotes. Indeed, molecular biology as a field of inquiry has been spectacularly successful in the past decade, and we are experiencing a new golden age of discovery. Yet despite our newfound abilities, in many ways I think that it is more difficult and a lot less enjoyable to carry out and publish seminal work in 2014 than it was in the early 1970s. The United States is now at a time when many question the relevance of science and the impartiality of scientists. Until the U.S. public demands adequate funding for a robust scientific enterprise, it seems unlikely that the pursuit of a scientific career will become once again a goal of our brightest young minds.

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