ASPB Pioneer Member

Philip Benfey

How did my career get started and where did I spend it?

When I was young, I didn't dream of becoming a biologist. I wanted to write the great American novel. But first, I needed a story to tell. And I had a feeling that college wouldn't be the place to find it. I dropped out after my first year, and drove cross-country in search of real-life experience. I found a job as a scaler for a logging company in Oregon. The primary skill required was adding three-digit numbers in one's head while walking along felled trees in order to determine the number of board feet in a log. I soon graduated to heavy equipment operator, which served me well in my next job, plowing the parking lots of a ski area in Utah.

When the ski area closed in April, I faced the decision of going back to college or continuing my wandering. I decided to hitchhike around the world on sailboats. Unfortunately, I kept hitting hurricane season, but I still managed to make my way around the world, working as a mechanic in American Samoa, as a bricklayer's laborer in Melbourne and on a track maintenance crew in Western Australia. This last job became more of an adventure than I had bargained for. I found myself in a test of wills between the Serbian line crew I was working with and our Croatian bosses. After calling a wildcat strike to insist that the safety regulations be followed, I was told that my life

was in jeopardy. I faced the ethical dilemma of either staying to help my fellow crew members or saving my life and opted for the latter.

My travels then took me to Indonesia, where I contracted amoebic dysentery, the Philippines, where I worked in the film industry and Japan where I spent a year and a half building Japanese gardens. After crossing the USSR on the Trans-Siberian Railroad, I ended up in Paris where the only job I could find was helping construct a community center on a barge moored in the Seine. I also met and fell in love with a woman who was in law school and is now my wife of 43 years. This seemed to augur well – I could continue my writing efforts, while she earned a good living as a lawyer. The pipe dream was shattered when she told me that she really wanted to pursue an acting career and that maybe I should find a day job. Although having never taken high school or college biology courses I had become interested in biology when I attended a conference in Japan on the origin of life. Working as a technician in a lab seemed a good option, but for that I would have to finish college. Getting admitted to a French University was not straightforward, given that I'd only completed a single year of college in the US. The equivalent of a French high school diploma was two years of American college. Fortunately, one of the three universities to which I applied agreed to bend the rule. The curriculum was entirely science-based, which allowed me to graduate in two years and convince the graduate programs I applied to that I had completed the equivalent of four years of an American college. It probably helped that I was at the top of my university class for both years. This I attribute to the fact that I was highly motivated, and it was a very interesting time to be studying biology. Recombinant DNA techniques were just starting to be used, allowing the identification of genes and their functions.

My Ph D. thesis advisor at Harvard, Phil Leder, was among the first to clone and sequence human immunoglobulin genes. From their sequences, he deduced that they are formed through successive recombination events, which explained how antibodies could be generated that are specific to a wide range of antigens. My initial project was to clone the receptor for IgE, the antibody that mediates the allergic response. Having

continued on next page

Philip Benfey ***continued***

suffered from allergies most of my life, I was personally invested. After extracting mRNA from 2,000 roller bottles of cell culture, it became evident that the receptor was made up of multiple subunits, and injection of RNA into frog oocytes was not the appropriate means of identifying receptor activity. Although disappointing, this experimental fiasco did teach me a key lesson in science: that one's passion for a project does not necessarily correlate with its success. While the receptor was eventually cloned in the Leder lab utilizing other approaches, my thesis project shifted to another aspect of the allergic response. Using published protein sequence, I cloned and sequenced two protease genes, each one specific to a subclass of mast cells, which are the effector cells of the allergic response. While completing the thesis, I began to look around for an area in which to pursue a post-doc. Analysis of the subclasses of mast cells piqued my interest in how cells acquire their identities. I looked into several biological systems: flies, worms and plants. The fly field seemed already quite competitive. When I asked Phil Leder about worms, he said that scientists working on worms claimed to be investigating behavior, but what they cloned was actin (this was the case for the first uncoordinated mutants). This left me with plants. Plants seemed attractive from a developmental biology perspective, because they appeared to have a relatively small number of cell types and the cells didn't move in relation to each other.

While in Boston, my wife acted in and directed theater productions, but saw more opportunities in film. She was admitted to the MFA program in film at Columbia, and I found a post-doctoral mentor, Nam-Hai Chua, at Rockefeller University in New York. When I arrived in Nam's lab, he suggested two projects. One was a bread-and-butter project to identify features of the 35S promoter, which was the most commonly-used constitutive promoter in plant biotechnology. The other was the more exciting project of making minichromosomes starting from a circular DNA virus that infects plants. The second project quickly crashed and burned. To analyze the 35S promoter, I used a newly introduced enzymatic marker, beta-glucuronidase. Addition of a substrate results in production of a blue dye that can mark specific cells. When I dissected this constitutive promoter into five pieces and placed them in front of a minimal promoter driving the enzyme, I was surprised to see that each piece conferred expression in a different set of cells. Moreover, when two or more pieces were juxtaposed, the resulting expression pattern was frequently more than the sum of the parts. These findings were published in five papers, and were an important lesson: there isn't a good correlation between what appears to be a bread-and-butter project and the ultimate results.

Many of the specific expression patterns could be seen in root sections. Observing these led me to an appreciation of the root as a model for developmental studies. A stem cell population resides at the tip of the root, from which all other cells are formed. Because cells don't move, all the different stages of development can be found along the length of the root, with the youngest cells at the tip and oldest toward the shoot. Moreover, a cross section of the root exhibits radial symmetry. Thus, the four-dimensional problem of development - three spatial dimensions and time - is reduced to two dimensions, with cell type on the radial axis and developmental stage on the longitudinal axis.

The plants being studied in Nam's lab at the time, tobacco and petunia, were not particularly amenable to genetic analysis. A community was forming around the use of Arabidopsis as a genetic model akin to drosophila and C. elegans. In all three systems, identifying the gene responsible for a mutant phenotype was time-consuming, consisting of the laborious process of chromosome walking. At a scientific conference, I met Ken Feldmann who had generated a large collection of insertional mutants in Arabidopsis. He was kind enough to allow me to screen the collection for root mutants. When I moved to NYU to set up my independent lab, I decided to focus on root development. From our mutant screens, the easiest pheno-

continued on next page

Philip Benfey ***continued***

types to identify were shorter or fatter roots. Close inspection of two of the mutants with shorter roots indicated that they were missing an entire cell layer. One mutant we had already named *shortroot*, and for the other we chose *scarecrow*, based on the character in the Wizard of Oz. Because the mutant phenotypes were caused by insertion of a known piece of DNA, we were able to clone the gene involved much more rapidly than by chromosome walking. The genes turned out to be founding members of the GRAS family of plant-specific transcription factors.

Thirty years later we are still working on SHORTROOT and SCARECROW and the gene networks they control. The remarkable series of technological advances that revolutionized biological research in this time span greatly aided our efforts. My lab has benefitted from enhanced imaging modalities, including confocal and light-sheet microscopy, as well as genomic technologies, including microarrays and short-read sequencing. We pioneered the use of fluorescent activated cell sorting of marker lines combined with microarray analysis to produce cell-type specific expression maps of the root. These techniques have now been complemented by single cell expression analysis, allowing integration of both cell type and developmental stage for every cell in the root. In addition to our long-term interest in cell specification, we have identified an oscillatory process by which lateral roots are positioned along the primary root of Arabidopsis. We have also branched out to other plant models, analyzing root formation in rice and maize. We have become particularly interested in understanding how in rice and other plants the root tip rotates 360 degrees as it makes its way through soil. This and much of our work has been performed in collaboration with other scientists around the world, which has enriched our science and made it substantially more productive. Of course, none of this work would have been possible without the generous support of government agencies like the NIH and NSF as well as, more recently, the Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation.

In 2002, I moved my lab to Duke, where I became chair of the Biology Department. Through a series of chance encounters I met a number of scientists from quantitative departments (physics, math, computer science, engineering) who expressed an interest in finding out more about biology. We set up an informal group to discuss biological networks. From those discussions emerged several collaborations aimed at using modeling approaches to address gene regulatory problems. When the NIH announced their intention to fund a set of centers to use and train others in the methods of systems biology, we applied and, much to our surprise, we were named the Duke Center for Systems Biology. I ended up running it for 6 years after stepping down as department chair.

At about the same time, I founded a company based on the technology we developed, which enabled gene expression in 64 roots to be visualized in real time. We used the "RootArray" as an assay for promoter elements that could drive expression in different cell types. The success of the company was the result of a combination of outstanding teamwork, very able people, and good luck. Within five years of operation, it was purchased by a major agricultural company.

When I started in biology about forty years ago, a fellow graduate student's thesis consisted of sequencing one gene. That an entire human genome can now be sequenced in a few hours is one measure of how much has been accomplished. I feel fortunate to have stumbled into a field that has seen such remarkable progress (and grateful to my wife for suggesting that I do so). I doubt very much that a career as a writer would have been half as interesting as the one that I've had.

What do you consider your most important contributions to plant biology?

If I have any lasting legacy, it will be in the people I have trained over the years. It has been a privilege to work with such intelligent, dedicated individuals, and to see them gain confidence in their abilities

continued on next page

Philip Benfey ***continued***

to design, execute and interpret experiments. I have tried to help them see that they have the ability to go out on their own, whether it be in academia, industry or the public sector. One of my goals has been to teach not only how to do good science, but also to manage issues such as leadership and group dynamics that determine workplace culture. Nothing gives me greater pleasure than to witness the success of my trainees both in their careers and in their personal lives.

What advice would you offer to a young person considering a career as a plant scientist.

Most of the critical issues facing the world today relate to plants. Climate change, which is caused primarily by burning the fossilized remains of plants, represents an existential challenge. At the same time, we face issues of food, energy, and water security, which also relate to plants and their uses. Research in plant biology can help address these issues. High on the list of priorities is understanding how plants can adapt to a changing climate and how they can mitigate climate change by reducing greenhouse gas emissions. Another challenge is how to properly communicate the goals and accomplishments of plant science to the public to avoid the kind of controversies that surrounded the first generation of engineered crops. Studying plants can also inform developmental studies of animals and even human disease. Plants and animals evolved independently with their last common ancestor being unicellular. The remarkable similarity in strategies used to go from stem cells to differentiated tissues in plants and animals suggests that there are limited ways to evolve a multicellular organism.