ASPB Pioneer Member

Richard Jorgensen

Following a strong high school education in mathematics and physics (but ironically no chemistry or biology), I entered the Engineering School at Northwestern University, where I initially majored in nuclear engineering, then moved to electrical and then chemical engineering, before landing in a new program in biomedical engineering and a four year BS/MS Chemistry program. In my junior year, I took my first ever biology courses—Bacteriology and Botany—as well as graduate level Biochemistry, half of which was molecular biology. Yes, I spent my time in college trying to find myself.

With an engineer's understanding of regulatory control, I was immediately excited by my first encounter with the concept of gene regulation, through an introduction to the lactose operon in Bacteriology class. From then on, DNA, RNA, and the control of gene expression were at the center of my intellectual universe. I quickly became a 'lab rat' for the final 12 months of college (missing a lot of classes, oops) in the laboratory of Professor Robert L. Letsinger, an early pioneer in nucleic acid synthesis and sequencing, learning to synthesize phosphoramidate ribonucleotides and incorporate them into RNA using RNA polymerase. Then, I was lucky enough to be admitted to the Ph.D. program in the Dept. of Biochemistry at the University of Wisconsin and be taken under the wing of a lac operon pioneer, William S. Reznikoff, who in collaboration with John Abelson's lab at UC San Diego was determining the first-ever promoter sequence, the lac operon promoter, by "wandering spot" chromatography. (Yep, that's how nucleic acids were sequenced back then!) "Rez" suggested for my thesis project that I sequence the lac repressor gene's promoter. I hopped to it, learning bacteriophage genetics to define deletions flanking the promoter. I decided on a new approach using restriction enzymes to identify a fragment carrying the weak RNA polymerase binding site of the lac repressor gene, but soon learned from Rez that Debbie Steege at Yale was miles ahead of me and would be using the new Maxam-Gilbert DNA sequencing method. Undaunted, and thanks to our next door lab neighbors, Douglas Berg and Julian Davies, I shifted gears and identified promoters in bacterial plasmids, settling on antibiotic resistance and transposase genes in the newly discovered transposable elements Tn5 and Tn10.

I next turned my attention to finding a postdoctoral position in plant molecular genetics, an emerging field in 1978, and landed in William F. Thompson's lab at the Carnegie Institute on the Stanford campus. I was not at all certain what research I would pursue, but I was intent on learning as much as I could about the molecular genetic nature of plants. In many ways, from genome organization and evolution to phytochrome control of development to physiological ecology, Carnegie was an ideal environment for learning. I soon learned that Carnegie was where the field of ecological genetics was created and developed by Clausen, Keck, and Hiesey, all dead and gone long before I arrived. But their protégé, Malcolm Nobs, was still around and introduced me to the wonders of *Mimulus* (monkey flowers), which I collected all over California and bred to recapitulate the principles of genetic coherence in populations and species. I soon realized that to really understand the molecular basis of evolution, I needed to embrace population genetics, and so I moved to a second postdoc in Robert W. Allard's lab at UC Davis, where I applied RFLPs to populations of cultivated barley and wild oats. I was poised to become a population and evolutionary geneticist.

But something funny happened in 1983: labs in St. Louis, Ghent

continued on next page

ASPB Pioneer Member

Richard Jorgensen *continued*

and Cambridge (UK) simultaneously developed the ability to use Agrobacterium to transfer genes into plants. Having been trained in bacterial genetics at the dawn of recombinant DNA technology, I immediately understood this technology would change everything. It would be possible to manipulate gene regulation - take it apart and put it back together - for the purpose of understanding how plants control expression of their genes during growth and development, and ultimately for understanding how plant genomes evolved to produce the array of diversity that defines the plant kingdom. I felt I just had to get back to regulatory control.

To exploit this opportunity, I answered an ad in *Science* magazine for a position with the eminent maize gene regulatory geneticist, Hugo Dooner, at a biotech startup called Advanced Genetic Sciences, Inc., which had been founded by John Bedbrook and others with the goal of engineering plant gene expression for crop improvement. John brought together an amazing array of plant scientists who were or would become true pioneers, including Hugo Dooner, Caroline Dean, Jonathan Jones, Gareth Warren, and Pal Maliga, among others. Yet again, I found myself afforded with a tremendous learning experience and the opportunity to do cutting edge research, this time with the goal of improving crop plants. My first project built on the knowledge of marker genes and sex expression I gained at UC Davis, and it led to patents for using engineered genetic markers linked to nuclear male sterility genes for hybrid seed production.

In 1986, Carolyn Napoli and I were married. Looking for a new direction in science, she decided to join AGS too, and together, building on our colleague Hugo Dooner's deep knowledge of flavonoid and anthocyanin genetics, we developed a program for engineering floriculture crops, such as chrysanthemums, carnations, and roses, with an emphasis on flower color. The most exciting and surprising result leveraged Hugo's observation that, in some plants, chalcone synthase (CHS) was rate limiting for flavonoid and anthocyanin biosynthesis. We postulated that if we engineered overexpression of CHS, we might deepen flower color. Carolyn engineered a petunia cDNA clone for optimal transcription and translation, using constructs developed by our colleague Jonathan Jones in John Bedbrook's and Pam Dunsmuir's lab. As an aside, Carolyn also engineered an antisense construct, but the true experiment of interest to us was the sense overexpression construct. To our amazement, the sense construct produced petunias with pure white flowers, as well as flowers with striking developmentally controlled patterns of white on an anthocyanin background. Was this due to an accidental mutant CHS subunit poisoning a CHS dimer or tetramer? No, the coding sequence was normal and unaltered. Incredibly, we soon found that BOTH the introduced CHS overexpression transgene and the homologous, endogenous CHS gene were coordinately suppressed at the RNA level. This was confirmed in parallel with two petunia anthocyanin genes by our Dutch colleague Alexander van der Krol in Jos Mol's lab in Amsterdam. We called this observation "co-suppression", filed a patent application in 1989 for silencing endogenous plant genes and published back-to-back with our Dutch colleagues in The Plant Cell in 1990. This research created quite a stir in the plant molecular biology and genetics communities (still largely separate at the time), especially at a Keystone Conference where the Editor-in-Chief, Bob Goldberg, distributed many dozens of copies of that issue with our petunia on the cover. We were mobbed at our poster.

Carolyn had recently accepted a faculty position at UC Davis (UCD) and embarked on developing a program investigating the developmental genetics of branching control in petunia. Months later, I decided to join her; John Bedbrook graciously arranged for me to be able to take the petunia co-suppression project, as well as a generous severance package to tide me over until I could obtain a research grant. UCD gave me a title (an unfunded 'soft money' position) so I could apply for grants. I acquired a USDA grant, but it was too small to do much more than partially pay

continued on next page

Richard Jorgensen *continued*

a salary and carry out epigenetic experiments in the greenhouse (molecular experiments were too expensive). To my shock and amazement, Japan's Ministry of Agriculture soon arrived at UCD and wanted to fund my research. There was enough new money to buy equipment and hire a postdoctoral, first Jim English, followed soon by Que Qiudeng, and a tissue culture specialist, Wang Huai-Yu. Carolyn generously allowed us to use her lab space and continued to collaborate on co-suppression.

Hugo Dooner had first pointed out to us the possible similarity of co-suppression to Alexander Brink's phenomenon of paramutation in maize, so I read up on it and reintroduced myself to Barbara McClintock's epigenetic discoveries. Long story short, at UCD I was able to show that our petunias exhibited not only co-suppression, but also essentially all of the phenomenology of paramutation, as well as the "setting" and "erasure" of heritable "epigenetic" states of gene expression discovered by McClintock (Brink called all such chromosomal states of gene expression "paragenetic", to distinguish them from the non-heritable, non-chromosomal "epigenetic" states that had been defined by plant cell culture biologists 60 years ago).

Experiments investigating the mechanism of co-suppression, carried out mostly by Que Qiudeng, showed that (1) truly single copy sense CHS transgenes could efficiently trigger co-suppression of endogenous CHS genes (more so than the corresponding antisense transgenes), (2) that translation of single-copy sense CHS transgenes was necessary for co-suppression, (3) that high level transcription of the transgene was necessary, and (4) that CHS enzyme activity was not necessary for the sense transgene to trigger co-suppression.

It is important to note that we did not discover RNA interference, though it turned out that co-suppression was indeed a version of RNAi. So, why did plant geneticists not discover that double-stranded RNAi is the trigger of RNAi? The reason was simply that we were all introducing DNA to plant cells, not RNA, and so we could only infer indirectly what might be happening at the RNA level. Some people did propose that dsRNA might be the trigger of RNA silencing, including my postdoc, Que Qiudeng, who intercrossed petunias carrying sense and antisense CHS transgenes and found the hybrids to be totally silenced. He proposed the simplest hypothesis that sense and antisense mRNAs interacted to form dsRNA. Peter Waterhouse at CSIRO also proposed dsRNA to be the trigger, but neither could he rule out a "homology sensing" interaction at the DNA level, the sort known in yeast and Drosophila, leaving RNAi to be proven directly in nematodes by Fire and Mello, who apparently were unaware of the plant research on gene silencing. Simply put, injecting RNA into worms was experimentally straightforward, whereas injecting RNA into plant cells was much more difficult, due to their tough cell walls. My UC Davis colleague, Bill Lucas, was the world's expert on that topic, and he was indeed keen to do the experiments before the Fire and Mello work was known publicly, though by then Carolyn and I had moved on to the University of Arizona. However, Bill and I continued to discuss aspects of RNA silencing and published a seminal hypothesis paper elaborating the existence of an "RNA information superhighway" in plants based on phloem and plasmodesmal transport.

Brian Larkins, Vicki Chandler, and Rob Leonard (the best Department Head I have ever known) were responsible for bringing us to Tucson. I have to say that it was quite an honor, being so valued by these three. And for the first time in my career, I had a firm platform on which to build a research program. While continuing work on co-suppression in petunias, I had become more interested in the "paragenetic" aspects of the system. This led me consider a reverse genetic approach, using RNAi to silence chromatin encoding genes in Arabidopsis, for which the complete genome sequence was being determined. Joining forces with my maize genetics colleague, Vicki Chandler, we designed a large-scale project for NSF's new Plant Genome Research Program (PRGP), assembled a team of ten PIs from around the country, and obtained a $12M 5-year PGRP grant.

continued on next page

ASPB Pioneer Member

Richard Jorgensen *continued*

The PGRP program was unique in creating the basis for developing a true community of plant scientists through multi-institution, collaborative research grants. Little did I know it at first, but leading a large PGRP grant positioned me well for participating in the development of a scientific consortium, and indeed developing such a community gradually became my primary scientific endeavor over the next ten years. Coincidentally, I was asked by Ralph Quatrano to join the editorial board of *The Plant Cell*, and then three years later the society appointed me the next Editor-in-Chief, my first true leadership role in the field of plant biology, something to which I had never really aspired, but I embraced it enthusiastically, feeling the time was right.

The Plant Cell was 15 years young, and I took the opportunity to reassess its position and future in the field. Transparency in reviewing became my first objective and my first decision was to require Editors to sign their decision letters. I also set a loose limit of five years service on the editorial board, expanded the scope of the journal, and appointed a more diverse and inclusive editorial board - scientifically, internationally, etc. The journal grew to 1000 submissions/resubmissions annually, with an editorial board of over 30 editors. The editorial board worked hard to obtain fair, detailed reviews, and to explain carefully to authors not only why their manuscript was being rejected or recommended for resubmission (if it was), but also what criteria needed to be met to recommend a resubmission. Without sacrificing quality, our approach was to be transparent, informative and inclusive. Instead of emphasizing a low acceptance rate (a false measure of scientific quality), we emphasized a high overall acceptance rate when including resubmissions. *The Plant Cell* experience was a valuable lesson to me for building community, and it served me well in my next major venture in NSF's Plant Science Cyberinfrastructure Collaborative (PSCIC) program.

NSF designed the PSCIC program to be a large, multi-institutional collaboration between plant scientists and computational scientists, but led by plant scientists to ensure that whatever cyberinfrastructure was developed would be certain to serve the domain of plant scientists' needs. In the long term, NSF envisioned this cyberinfrastructure program would serve the biological sciences broadly. Plant science was chosen as the initial focus because plant scientists already functioned as a diverse, collaborative community and worked across diverse species, including model species and crops, and disciplines from molecular and developmental biology to evolution and ecology. At the time, the plant science community was unique in these respects, largely due to the PGRP, which funded large, multi-institutional research projects centered on genomics, which had become the central, unifying discipline of biology.

The University of Arizona led a multi-institutional proposal that successfully competed for the 5-year, $50M contract. We named it The iPlant Collaborative and I served as the PI and Founding Director. Essentially, NSF funded a team and a concept, and we proceeded to hold a series of workshops, mainly at the Biosphere-2 facility North of Tucson, bringing together a number of different, self-forming groups of plant and computational scientists to discuss cyberinfrastructure needs and develop plans for cyberinfrastructures that would serve the plant community's needs. The iPlant team coordinated these efforts, chose several of the best projects to initiate CI development, and set to work integrating the CI collaboratively with plant and computational scientists across the country and around the world. The iPlant Collabotive was successful in this respect, and as anticipated by NSF it evolved and expanded to serve diverse communities of biologists far beyond the scope of plant science, later changing its name to CyVerse to reflect this newer, broader mission. Prior to expanding iPlant's scope, and even before the iPlant's functional CI was developed, I decided to retire from the University of Arizona and leave iPlant's future development to the excellent team that had brought the plant and computational science communities together.

Subsequently, I assisted the faculty and directors of two plant biology research institutes, the

continued on next page

ASPB Pioneer Member

Richard Jorgensen *continued*

Laboratorio Nacional de Genómica para la Biodiversidad in Irapuato, Mexico, and the Carnegie Institute's Department of Plant Biology at Stanford, CA, where I had done my first postdoc. I also consulted on intellectual property, mainly regarding RNAi patents, primarily helping to defend the Fire and Mello (Carnegie and U Massachusetts) RNAi patent against an interference. In a sense, this brought me full circle, engaging both my academic and biotechnology industry interests. This was perhaps a fitting close to an unusual career that veered from biomedical engineering to molecular genetics, from academia to industry (and back), and from epigenetics to cyberinfrastructure.