Brian A. Larkins

How did you spend your career?

There was little about my experience growing up on a small farm in Southeast Nebraska that would have predicted a career as a plant scientist. By the time I was in high school, I was aware of two things: 1) I was never going to make it as a farmer; and 2) biology was my favorite subject. But when I explained my aspiration of majoring in biology at the University of Nebraska to my high school guidance counselor, he told me there were few jobs available for biology majors (forest ranger was his example), and I would be better off majoring in math. That way if I didn't like teaching, I could get a job as an actuary and work for an insurance company. Based on this advice, in the Fall of 1964 I enrolled as a math major in Teachers College at the University of Nebraska.

That summer when I received the registration for my courses, I was surprised to find I would be taking an introductory course in botany; I wasn't exactly sure what botany was, so I looked up the word in the dictionary and learned it was about plants, something that didn't receive much attention in my high school biology class. Although I never knew why a course in botany was selected for math majors, many years later I learned the undergraduate math advisor in Teachers College was enthusiastic about the way in which Professor John Davidson taught introductory botany, and it turned out to be a turning point in my life.

Botany 001 was unique in many ways. The first day of class we were told that if we had purchased a textbook and a lab book for the class, we should return them for a refund, as the class didn't use either of them. We were told the plant would be the source of information. Botany 001 was a four-hour course with two one-hour recitations and two three-hour labs each week. What we learned came from investigations in the lab; the recitations were used to present these discoveries and debate their interpretation. In the first lab we were given seeds and seedlings of peas, beans, castor beans, and corn, and we asked to compare and contrast their structures. This required that we ask for the names of their parts: leaves and stems wasn't sufficiently specific. After we sorted out the comparative morphology of these plants, Dr. Davidson asked what was the first thing to come out of the seed? It is the root. And so how does it get out, he asked? This led us to look at emerging root tips with a microscope and discover there were cells dividing. We proved that the cells were dividing and not fusing by measuring and counting them. The root tip squashes also showed us that once past tip, the cells enlarged and developed unusual cell walls – looking like train tracks. This led us on an exploration of how plant roots develop, and when we finished with the root, we started at the top of the stem and came down.

So, Botany 001 turned out to be a course in plant anatomy and plant development. We were discouraged, indeed warned, not to seek information in books. Any conclusions we made had to be based on observations in the lab: the plant is the final authority! Also, there were no exams! There were a few quizzes, but those grades didn't count. Your grade for the course was determined by your performance on the final exam. Dr. Davidson said that what mattered was what you knew by the end of the course. Clearly, a lot was riding on the final, and this freaked out some students – particularly the pre-meds. I did ok- I got a B+. Botany 002 was about plant evolution. In this class, we examined frozen or pickled specimens of plants, starting with algae and moving up to liverworts, ferns, pines and flowering plants. In the lab, we would draw key structures during the plant's development, and diagram it's life cycle. At the end of the course the challenge was to compare and contrast life cycles of the various plants and figure out what had changed

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progressing from a single celled plant to a flowering plant. In the process, we discovered the evolution of plants from single celled organisms (gametophytes) to multicellular, sexually reproducing flowering plants (sporophytes).

For me, these classes were an epiphany. I discovered that given the opportunity, I could use my brain and figure things out. Every other class I had taken involved learning facts or procedures and regurgitating them or using them to solve puzzles – like math problems. Dr. Davidson's teaching approach is called learning through "inquiry". It is also known as the "Socratic" method – attributed to the way in which Socrates is said to have taught. You draw out of the student things they know and help them to reason facts from them. This is not an efficient way to cover a lot of teaching material, but it is a very effective way to teach students to reason and understand what they know. I became a big fan of Dr. Davidson and his graduate students who taught the labs. They were smart, interesting, stimulating, and inspiring. I began thinking that I'd like to be a member of that group someday. I ended up majoring in biology, as I was spooked about the forest ranger warning. After I graduated, I taught high school biology for a year, but I found the students there were far less interested in learning biology than I was teaching it. Before the end of the year, I applied to be a graduate student in the Botany Department at the University of Nebraska.

The Ph.D. project my advisor, Eric Davies, gave me was an extension of his graduate work, which focused on the question of how plant cells grow. Eric had shown that auxin treatment of etiolated pea epicotyls caused the production of cellulase, which could cut cellulose fibers and thereby theoretically loosen the cell wall, allowing the cell to expand. He had done experiments showing that a preparation of ribosomes from auxin-treated pea seedings could generate detectable amounts of cellulase activity. But these data were crude, and my job was to refine the system by purifying polyribosomes that were translating cellulase mRNA and determine if they were increased by auxin treatment. However, it was very difficult to isolate intact polyribosomes. Once cells are broken during tissue homogenization, ribonucleases can quickly sever the mRNA holding ribosomes together. Through a bit of serendipity, I discovered that ribonuclease activity could be effectively blocked in a high pH Tris buffer. Within a year of starting the project, we published a manuscript in Plant Physiology describing a procedure for isolating intact plant polyribosomes. In the end, I didn't answer the question about the effect of auxin on cell wall expansion, but I published three papers on plant polysome isolation, and I envisioned a model system to study mRNAs and protein synthesis in plants: seed storage proteins.

After completing my PhD in 1974, I moved to the Botany and Plant Pathology Department at Purdue University for postdoctoral research with Arthur Dalby and Charles Tsai, working on seed storage protein synthesis in maize. This research proved very successful, and in 1975 I was hired as a faculty member in that department. I spent the next 10 years doing research on maize seed storage protein synthesis and progressing through the professorial ranks. This was an exciting time, as traditionally trained plant scientists, including me, were learning how to apply molecular biology and gene cloning techniques to their research projects. Besides chloroplasts, mitochondria, and *Agrobacterium tumefaciens* induction of plant tumors, seed storage proteins were model systems and the focus of many national and international meetings.

In 1988, I was recruited to be Head of the Department of Plant Sciences at the University of Arizona, where I had the opportunity to build an agricultural department that integrated molecular biology into its teaching and research programs. This was a novel concept at the time, and it proved to be a greater challenge than I had imagined. After six years as department head, I spent the next 18 years teaching and doing research in the Plant Sciences Department. When I retired from the University of Arizona in 2012, I was invited to become Associate Vice Chancellor for Life Sciences at my alma mater, the University of Nebraska. In this position I had multiple challenges, including coaxing the deans and department heads of four different colleges to collaborate in their life science

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teaching and research programs and create interdisciplinary degree programs. This too turned out to be more challenging than I imagined!

My research program at Purdue and the University of Arizona focused on the regulation of seed development and the synthesis of seed storage proteins. Storage proteins are the most abundant proteins in seeds, and as such they are the principal determinants of the protein quality of grains. Storage proteins are generally deficient in several amino acids that are required in human and livestock diets. Consequently, increasing the levels of these essential amino acids has long been a goal of plant breeders and cereal chemists. A major focus of my research was Quality Protein Maize, or QPM. The *opaque2* mutation increases the content of essential amino acids in the maize kernel, but it also causes a soft starchy endosperm that creates inferior grain quality. Genetic suppressors of *opaque2* (*o2* modifiers) were identified that ameliorate the negative phenotypic features of the *o2* mutation, but the genes responsible for modification were not well characterized. We studied how the *o2* mutation increases the lysine content of the grain and how *o2* modifiers restore the normal hard, vitreous kernel phenotype. We also investigated cell cycle regulation, particularly the process of endoreduplication, and the role it plays in endosperm development.

What do you consider to be your most important contributions to plant science?

One of my most valuable contributions was the development of methods to isolate intact polyribosomes from plant cells. In the 1970s, it was very difficult to isolate mRNAs. The poly(A) tail at their 3′ end had not been discovered, and the best option to isolate mRNAs was through their association with ribosomes. Polyribosomes, mRNAs attached to multiple ribosomes, could be isolated by ultracentrifugation. However, once cells are broken open, the ribonucleases released can quickly sever mRNAs, turning polyribosomes into ribosomes attached to mRNA fragments. DNA isolation was much easier! I discovered that high concentrations of highly alkaline Tris buffer (pH 8.5) was sufficient to block RNase activity in tissue homogenates. I also found that divalent ions, such as calcium, which is abundant in some plant tissues, can greatly reduce recovery of large polysomes. This problem could be solved by including small amounts of EGTA (ethylene glycol-bis (β-aminoethyl ether), a strong chelator of calcium, in the Tris buffer. Within a short time after arriving at Purdue, Andy Jackson (a plant pathologist) and I were able to show that an EGTA-containing Tris buffer was effective for isolating plant viral mRNAs from infected leaf tissue. This turned out to be effective method for polysome isolation from a variety of animal tissues as well. These discoveries were valuable for my own work, as well as that of many others during the early years of plant molecular biology research.

My other contribution was applying molecular genetic techniques to plant biology research. Within a short time of starting my postdoctoral work at Purdue, I was able to isolate zein-synthesizing polyribosomes from developing maize kernels. When placed in a cell-free protein synthesis system made from wheat germ, they produced zein proteins *n vitro*. Subsequently, I isolated zein mRNAs, and we used them to clone zein genes – among the first plant genes to be characterized. Later we showed that the poly(A) tail on zein mRNAs was less important for their stability than it was for their translational efficiency- something that turned out to be universally true of mRNA poly(A) tails. Seed storage proteins were a valuable model systems for plant molecular biology research, and this research laid the foundation for subsequent studies of plant growth and development.

When did you become a member of ASPP/ASPB?

I became a member of ASPP in 1972, when I attended the annual meeting in Calgary, Alberta. I was there with Eric Davies, my PhD advisor, and another graduate student, Joe Waldrum. We were newcomers to ASPP and hardly knew anyone there. But we met many people at the opening reception, an “open bar” funded by the Canadian Society of Plant Physiology. Before the night was over, we had made many new friends. It was impressive to see the famous plant

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physiologists there who I only knew only from their publications: Hans Kende, Anton Lang, Joe Key, Tony Trewavas, and Derek Bewley among them. I gave a talk on my polysome research: I was so nervous I made multiple trips to the bathroom the hour before the session began! It was valuable experience, and it launched my career.

How did the society impact your career and what was your motivation for becoming a Founding Member of the Legacy Society?

ASPB had a major impact on my career. The society's journals were an effective and inexpensive way to publish the research coming from my lab. The annual meetings provided opportunities to meet with colleagues, and competitors, and learn about their research. I established many research collaborations at these meetings. The mini-symposia described cutting edge science and frequently were a source of inspiration for new research projects. The annual meetings were an opportunity to gain a global perspective on plant biology and a sense of what research agencies were funding. The meeting also provided an opportunity to recruit new postdocs and faculty members. I was provided the opportunity to serve on editorial boards for Plant Physiology and The Plant Cell, as Editor-in-Chief of The Plant Cell, and eventually as President of ASPP. This was a fair amount of work, but it was certainly an educational and a rewarding experience! Serving on a variety of ASPP committees created many professional friends and provided me a chance to serve the society that did so much for my career. So, of course I didn't hesitate to become a member of the Legacy Society

What important advice would you give to individuals at the start of their career in plant science?

Becoming a professional plant scientist requires much time and a lot of work, but if you love biology and are curious and creative you will find it to be a wonderful career and a great way to spend your life. The plant community is smaller and less competitive than the animal/medical community, and you will find teachers and colleagues that support you throughout your career. The following is the short and simple advice I give to graduate students and postdocs: 1) Never miss an opportunity at public speaking; 2) Begin a collaboration with your major competitor – that way you'll know what he or she is doing and they can't review your grant proposals; 3) Competition is your friend; 4) Follow your passion, but recognize an opportunity when it presents itself.; 5) Don't be the smartest person in your lab; and 6) Create a work environment that maximizes your student's and postdocs innate abilities- if they are successful, you'll be successful.

Academic Family Tree
https://academictree.org/plantbio/tree.php?pid=632939