Edgar Spalding

How did you spend your career?

The seed of my career germinated in 1981. My first biology professor at Acadia University, George Curry, studied phototropism with Kenneth Thimann in the late 1950s. In class, Curry used the process of constructing an action spectrum (his specialty) to demonstrate how a piece of biology could be understood by applying first principles from physics and chemistry. That strongly appealed to me. Pow! I wanted to be a plant photobiologist.

My project in Curry's little lab showed that UV-B radiation promoted the emergence of the primary leaf through the tip of the coleoptile during seedling establishment, an example of beneficial photomorphogenesis induced by wavelengths typically associated with causing damage. Curry encouraged me to go to graduate school. He thought I should try for a Rhode's Scholarship and attend Oxford. I applied, was a finalist, but not selected. Thank goodness, as it turns out.

How should I become a plant photobiologist? In those days, believe it or not, the Smithsonian Institution operated a research center that specialized in plant photobiology. Dr. Walter Shropshire was a principal investigator there and a professor at George Washington University. Dr. Shropshire published 'the other' phototropism action spectrum shortly after Curry's. A letter of introduction from Curry paved the way for me to meet Shropshire during a visit to Washington, D.C. where my grandparents and some other family lived. With Shropshire I had my first professional discussion about photoreceptors. I held my own and it was exciting. Shropshire explained that he couldn't take on a new graduate student but, "I have just come back from Penn State. Dan Cosgrove is looking for a new student. Shall we call him?" Curry had shown me one of Cosgrove's papers on the rapid inhibition of hypocotyl growth by blue light and a short piece about Dan including his picture in the newsletter of the American Society of Photobiologists. In my mind, I already knew him. Shropshire picked up the phone and said "Hi Dan, it's Walt. I have a young fellow here in my office..." Dan and I chatted. Four months later, I dragged my suitcase off a Greyhound bus in State College and started looking for someone with a beard. Alas, Dan had shaved, but all worked out. It was July 1985.

Dan used blue light as a fast-acting growth inhibitor to help him determine that wall yielding and not any hydraulic parameter was the predominant control point for cell expansion. I soaked up the biophysical thinking and 'gadgeteering' in the lab while I eagerly took courses like Physical Chemistry, Intro Quantum Physics, Electrical Circuits, Membrane Biology, Advanced Plant Physiology, and Plant Anatomy. I essentially flunked Differential Equations and Molecular Biology. I also flunked my first research project, which was to fabricate micron-scale pH electrodes and use them to measure apoplastic pH during blue light-inhibited growth in etiolated cucumber hypocotyls. I was a frustrated, impetuous 22-year-old when I told Dan that I needed to give up and be assigned another project. "Go to the library", was his reply. Off I went, grumbling. My fortunes and attitude turned around. I found my own path to a different but related electrophysiology project. When it was time to leave the nest, Dan wisely suggested that I find a postdoc position where I would learn new methodologies. Patch-clamping was then (1990) revolutionizing membrane transport research. Dr. Mary Helen Goldsmith at Yale was one of only a few plant biologists who could provide training in the difficult method. She offered me a postdoc position in an arrangement that included an association with Clifford Slayman (School of Medicine) and his talented postdoc, Adam Bertl. I soaked up membrane transport theory (along with plenty of beer) and learned technical tricks from Adam. Mary Helen intended me to study potassium channels in growing oat leaf cells, but I felt that doing the same kind of work in

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Arabidopsis could have more impact. She generously indulged me.

After some success over three years at Yale, I started looking for a faculty position. I was fortunate to be offered the position vacated when Ken Keegstra left the University of Wisconsin. When I arrived in Madison, Folke Skoog huffed, "How big are your feet, boy?" which left me shaken, as intended, but undeterred. My first goal in 1994 was to combine electrophysiology and photobiology in studies of Arabidopsis seedling growth. In what now seems like the blink of an eye, my new group was studying potassium nutrition, auxin transport, and glutamate receptors using electrophysiology and molecular genetics. We got good at measuring growth by digital image capture and analysis and began to practice what is now called high throughput phenotyping. Influenced by my colleagues in Agronomy, our interests eventually expanded to include measurement challenges in maize research and breeding. Oh, in case my Dean reads this, I teach every year – at least half of a large introductory botany course and a full course in plant physiology with a lab.

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

1. One day in the darkroom at Penn State, I attached two surface contact electrodes to a growing cucumber hypocotyl, roughly following an old paper I found in the library stacks. I shaded the basal contact zone and irradiated the apical one with blue light. After a lag time of a few seconds, a beautifully smooth and large voltage transient drew out across the chart recorder. Huh? I hooked up a new seedling and gave red light first. No response. Then I gave blue light and watched the same beautiful curve unfold. My pulse was racing. Blue light induced something that smacked of plasma membrane ion transport a few seconds before growth dramatically slows. I knew I had something PhD-worthy to figure out, and to a significant extent I did. At Wisconsin, I transferred this work to tiny Arabidopsis seedlings, which required the development of computerized image analysis methods to measure growth rate. We identified the effective photoreceptors, characterized a blue light-activated anion channel, found a potent blocker of it, found a membrane transporter gene that the blocker induced, which led to the discovery of ABCB transporters that transport auxin in a channel-like fashion. Some of these things are in the textbooks today.
2. Mike Sussman's group knocked out the AKT1 channel in the early days of Arabidopsis reverse genetics. By patch clamping, we showed that the mutant's root cells completely lacked inward-rectifying potassium channels in the plasma membrane. Sussman's group showed that the mutant displayed poor growth in very low (10 μM) potassium conditions. Conventional wisdom said that a channel could not take up potassium from a 10 μM solution because that transport would be energetically uphill. One direct measurement reconciled all the observations. Membrane potential was, surprisingly, more negative than the -236 mV that calculations showed would be necessary for channel mediated uptake in that condition. Thus, we had demonstrated that Arabidopsis takes up its most abundant metal against a ten-thousand-fold concentration gradient using a thermodynamically passive channel. *Science* featured this work on the cover in 1999. The conclusion is in textbooks today.
3. This one was born from my bad advice. I asked a student to use gluconate instead of chloride as the anion for some potassium shift experiments. When no potassium gluconate could be found, I told her the potassium glutamate on the shelf should do. The results she later showed me were physically impossible, unless...At that time (late 1990s), I would sometimes type 'channel' or some other equally clever query to look for I don't know what in the emerging genome sequences – ridiculously naïve but nonetheless helpful because evidence of sequences related to animal glutamate-gated channels was returned. Aha! The glutamate I suggested my student use as an impermeable and inert anion had activated a conductance for some ion that was very far from equilibrium. Had Ken

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Keegstra left behind some potassium gluconate on the shelves, we probably would not have shown that Arabidopsis *GLR* genes encode calcium channels gated by amino acids and glutathione. Our patch-clamp study of GLR3.4 showed it to be a serine-gated, calcium-selective channel. The 3D structure of GLR3.4 was recently published. It showed a serine molecule lodged in the expected ligand-binding cleft and a glutathione tucked into a novel site. It looks like we got things right.

4. I settled on computer-controlled CCD cameras that could record images in infrared light and wrote the rudimentary image analysis software for minute-by-minute measurements of Arabidopsis hypocotyl growth. I was smart enough to realize that with two cameras we could do twice as much. What would happen if we had many cameras? Well, I found out. First, I would need much more sophisticated computer sciences expertise, which Nathan Miller and Miron Livny supplied. With many cameras and high throughput computing we could treat phenotypes as time-dependent processes, not endpoints, and measure them in populations, not only single mutants. The result was that we added a high-resolution time axis to QTL maps for dynamic processes such as root gravitropism. We helped other groups adopt this way of working before there was a field called high throughput phenotyping.

How did the Society impact your career, and what motivated you to become a Founding Member of the Legacy Society?

ASP(P)B was a principal component of the environment in which I developed from student to professor. First, I learned from *Plant Physiology* how to comprehend a study. Curry would pass along each new issue of the journal to me. Later, the society's annual meeting was where I learned how to explain experiments with posters, at a podium, and in hallways. I learned what made our community effective as I served on the the society's Membership Committee, Program Committee, Executive Committee, Board of Trustees, *Plant Physiology* editorial board, Business Development Committee, and a term as Secretary. I learned the good a community can produce if some within it care enough to put thought and time into administration that creates opportunities for its members. Becoming a Founding Member of the Legacy Society is both an honor for me and a way to help make it possible for others to benefit as I have from ASPB.

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

1. Go to the library, so to speak. It is one place in this business that will never let you down.
2. Challenge your own thinking and assumptions. It will reduce the chances of you accepting a soft conclusion that you will later regret.
3. You may be an unreasonably harsh critic of your own work but not of others. Judge theirs as you would have them judge yours.