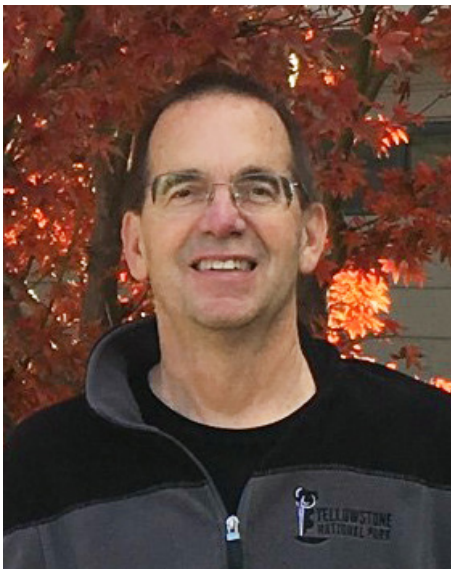
ASPB Pioneer Member

J. Clark Lagarias



How did you spend your career?

The seeds for my career in the plant sciences came from the woods behind my childhood home in Penn Hills, a Pittsburgh suburb, where I spent the lion's share of time in summer exploring the plant, animal, and mineral environment. The scent of hyacinth, which greeted me and my family upon entering the glass house at Phipps Conservatory, is unforgettable - along with the brilliant flower colors which cut through the sulfur smoke and grey blandness of the Pittsburgh winter. These memories, and fort building, sustained me throughout my early education in Pennsylvania and my high school years in Maryland. The fact I couldn't keep still in a chair led to early lessons in conformity, which I have never quite mastered, and contributed to my lack of interest in books I found hard to read, despite the efforts of remedial reading teachers. I always liked my science teachers best and was fortunate to have quite a few great ones growing up, in particular Mr. Diebler in 4th grade and Miss Simmons in 11th.

Since the present was suitably engaging to me, I never thought much about the future until I was nearing college age. To keep my career options open, I enrolled at the University of Michigan - one of the largest universities on the planet, where I chose to live in the intimate 'Residential College' to receive the benefits of a small college learning environment. I was a pre-med, like so many of my friends who favored science. However, the cold Michigan winter and my first roommate were so displeasing that I transferred to UC Berkeley after one semester. The palm trees and the redwoods in California became my new friends - my second college roommate, not so much. I chose chemistry as a major, because I was advised it would help me get into medical school, and after my first semester At UC Berkeley, I landed an undergraduate research position in an organic chemistry lab, which I loved.

After another year of large enrollment courses filled with other pre-meds and upon completing a general education 'practical' course called "The California Flora" that I especially enjoyed, I began to rethink my medical school plans and sought help from the vocational guidance office. To my astonishment, my personality assessment and vocational questionnaire indicated I was most suited for a career as an undertaker. Mission accomplished! It was then I signed up for a second major in botany. This turned out to be one of my best decisions, owing to the great botany instructors at UC Berkeley. I vowed to combine my interests in chemistry and botany to my choice of a career. Again, most of my classmates thought it was a big mistake to forsake medical school, so I kept this plan to myself.

I did end up applying to medical school and even got accepted. However, the call of chemistry was too strong, so I stayed on at UC Berkeley to seek a PhD in chemistry – again against the advice of my undergraduate advisors and peers. Although I had agreed to finish my undergraduate research project on an alkaloid natural product family, I negotiated 'free time' to develop a new project that bridged my interests in plants and organic chemistry and work on the structure and linkage of the light sensing chromophore of phytochrome. I had wanted to work on florigen, but I was advised not to by Anton Lang. That was the only advice I actually heeded, fortunately, since florigen wasn't identified until 30 years later and it turned out that florigen isn't a small molecule! My phytochrome project was the next best step toward my career goal, because of its role in mediating photoperiodic floral induction and all that came with flowers, i.e., their color diversity, amazing scents, and rich chemistry. During my PhD, I had amazing mentors - my undergrad mentor

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and colleague, Richard Houghten, my PhD advisor, Henry Rapoport, and two collaborators, Alex Glazer and Winslow Briggs. After I finished my PhD studies in the summer of 1979, I began the search for a post-doctoral position, which I had not even considered before then.

How I eventually ended up at UC Davis, where I have spent my entire career, is a short albeit convoluted story. Let's just say I owe my career path to great mentors, a bit of luck, the weather, my lack of planning and ignoring advice. At the outset, as a faculty member at UCD, I soon became overwhelmed. I owe my success to great friends, supportive colleagues, espresso, group therapy and finding my soulmate. The process of scientific research always was my safe place, but I was compelled to phase out my own involvement in experimental research after the first ten years - replacing it with teaching, grant writing, attending meetings and editing manuscripts. I learned to rely on daily updates from students and postdocs - failures and successes both - for my mental health. Although I do enjoy teaching, I remain uncomfortable speaking to large audiences, while one-on-one discussions with colleagues re-invigorate my spirit.

When I arrived at UC Davis in 1980, I knew I wanted to work on phytochromes. However, I soon learned that the hardest part of academic research is managing people. I had to evolve past my micromanager tendencies to realize that allowing my colleagues to own their failures and successes was more effective. That epiphany took me 20 years. Although my initial years were a bit bumpy, I was fortunate to have many exceptional students and postdoctoral fellows join the lab. During this apprentice period, my lab helped elucidate the biosynthetic pathway of the linear tetrapyrrole (bilin) pigment component of phytochrome using etiolated oat seedlings as the experimental material. This required us to harness radioisotope tracers, develop a coupled holo-phytochrome assembly assay, and leverage oat etioplasts to achieve >50,000-fold enrichment of phytochromobilin synthase - the penultimate enzyme in the synthesis of the phytochrome chromophore – all this without the benefit of commercial kits! One of my students purified phytochrome from lysed protoplasts of the streptophyte alga, *Mesotaenium caldariorum*. It is still the only 'green' plant species from which phytochrome has been purified to homogeneity, as the presence of chlorophyll precluded its purification except from etiolated plant tissue prior to, and since that time.

Although the first molecular cloning of a phytochrome cDNA from oats occurred in 1984, the new tools of molecular biology were not widely available to the phytochrome community at that time. In 1989, my laboratory leveraged the oat phytochrome A cDNA to confirm our earlier *in vitro* assembly studies showing that holo-phytochrome assembly was indeed autocatalytic, requiring no other factors than the bilin and the apoprotein. In the mid-1990s, we cloned the algal phytochrome gene by screening a genomic library using a long inosine-containing primer based on the protein sequence deduced by proteolysis and Edman degradation of the purified protein. In a rare return to the lab, I purified DNA for genomic library preparation from algal protoplasts using cesium chloride gradients. With both algal and plant phytochrome cDNA clones now in hand, we showed the recombinant phytochromes had photochemical and biochemical properties indistinguishable from those isolated from natural sources. The successful cloning of phytochrome synthase by Takayuki Kohchi in 2001 enabled our lab to clone and biochemically characterization the paralogous family of ferredoxin-dependent bilin reductases of cyanobacteria. These studies provided the final tools needed to reconstitute holo-phytochromes in *E. coli*, yeast and/or mammalian cells - a technology that has revolutionized structural, biochemical, photophysical, and optogenetic studies of phytochromes and their relatives as they were identified from DNA sequences in newly sequenced genomes. The first of these, determined by the Kazusa DNA Research Institute in Japan, was the cyanobacterial phytochrome 1 (Cph1) identified

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in the genome of *Synechocystis* sp. PCC 6803. These studies presaged discovery of the widespread distribution of phytochromes in prokaryotes as well as other non-photosynthetic and photosynthetic eukaryotic species.

By the end of the 20th century, other labs, utilizing genetic and reverse genetic approaches with the model plant *Arabidopsis thaliana*, were pursuing studies to identify the signaling pathways of phytochromes in plants. My group instead focused on the bilin chromophore and its interaction with the apoprotein by developing approaches to probe the structural basis of phytochrome's ability to switch between two photostates with red and far-red light. We leveraged synthetic organic chemistry, enzymology, directed evolution, and genetic diversity of the expanding phytochrome superfamily. Leveraging Cph1 as a model for plant phytochrome, together with functional studies with Arabidopsis phyB *in vivo*, our studies have proven effective at dissecting the structural basis of light sensing by phytochromes. The discovery that substitution of a conserved tyrosine residue with histidine generated a photoinactive Cph1 holoprotein that is intensely red fluorescent led to an even more exciting discovery, that the same mutation in Arabidopsis PHYB yielded a light-independent signaling active holoprotein - the first constitutively active allele of a plant phytochrome.

In the past twenty years, I have been fortunate to have collaborated with many outstanding individuals, but I particularly need to acknowledge my long-term associates: Ms. Shelley Martin, my lab manager, and two professional researchers, Nathan Rockwell and Wei Hu. It has been - and continues to be - a privilege and pleasure to work with them. Notably, during this time my laboratory extensively leveraged the genetic diversity of the phytochrome superfamily, which not only includes land plants but also encompasses the cyanobacteriochromes - a subfamily of bilin-based photoswitches found exclusively in cyanobacteria. Studies on cyanobacteriochromes revealed a plethora of tuning mechanisms whereby the protein governs the absorption spectrum and photochemical properties of their bilin chromophores. Such mechanistic insight revealed billions of years of molecular evolution that resulted in an unparalleled color sensing palette for this family, from the near UV through the near infrared. More recently, convergent evolution of phytochromes found in eukaryotic algae and aquatic plants has yielded a broad color palette within eukaryotes. We expect this knowledge will guide efforts to bypass the long process of natural evolution of phytochromes and create approaches to introduce novel light sensing variants into plants that will influence their acclimation to light environments not presently suited to crop species.

What are your most important contributions to plant science?

The people who I have mentored remain my most important contribution to plant science. It is these individuals who will uncover new knowledge for building upon the foundation already laid down by their predecessors. I have been privileged to have worked with many bright and committed individuals, many of whom have gone on to head their own labs in academia, industry, and broader society, and continue to spread the word of evidence-based decision making. I am heartened by their positive attitudes and strong ethics, notably their efforts to champion the under-served and to counter misinformation in society at large.

Other key contributions include the knowledge, tools and reagents that my lab has made available to the research community. Notable amongst these include phytochrome photochemical parameters used to calculate the degree of phytochrome activation under different light environments, identification of new photoreceptor variants with novel photochemical/fluorescent and functional properties, development of genetic-based tools for bilin biosynthesis to reconstitute/inhibit holophytochrome production in any genetically transformable species that are now widely used for optogenetic and plant trait modification applications, and Arabidopsis plant lines deficient in one or more phytochromes and/or in bilin chromophore

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biosynthesis used for genetic cross talk and photoreceptor complementation purposes.

Our research also has provided insight into the origins and evolution of phytochromes through studies on extant phytochromes from widely diverged lineages of photosynthetic species as well as non-photosynthetic eubacteria and eukaryotes. This knowledge revealed phytochrome's likely genesis as a light-and bilin-regulated two component histidine kinase with subsequent replacement, removal, or alteration of an ATP/GTP-dependent output signaling module. Since bilin biosynthesis depends on the presence of oxygen, phytochromes are integrators of both light and molecular oxygen levels. Our most recent studies also suggest a potentially more universal role of bilins as antioxidants that protect and sustain chlorophyll biosynthesis from photooxidative damage - a role that likely preceded the origin of the phytochromes during the genesis of oxygenic photosynthesis itself.

When did you become an ASPB member?

I joined ASPB during graduate school in the late 1970s, when I was working in Melvin Calvin's laboratory at UC Berkeley. This was a rich environment underwritten by the Department of Energy (formally the Atomic Energy Commission), consisting of plant biochemists, photophysical and organic chemists, and biophysicists - all of whom broadly focused on photosynthesis and energy conversion. The weekly colloquia were chaired by Melvin Calvin and a dozen senior staff - notables in the areas of photosynthesis, photoenergy conversion, origin of life studies, vision and the effects of radiation in genetic damage and cancer. While many of the topics of these seminars were indecipherable to me at first, over time I began to understand the questions and approaches that each research group were applying to their research problems. This introduction to interdisciplinary approaches to biological research influenced my entire career, while my ASPB membership connected me to the plant biology community at large.

How did the society impact your career?

As a member of ASPB, I have taken advantage of its outstanding plant biology journals, Plant Physiology and The Plant Cell, to communicate my laboratory's best plant biology research findings. Both journals have a sustained record of cutting-edge plant biology research curated by outstanding reviewers and discipline-trained editors. I also have made it a point to accept all review tasks for both journals when asked, and my service as a member of the reviewing editorial board for The Plant Cell in the last decade has been my attempt to give back to the society. I am very honored to being recognized as an ASPB Pioneer.

Owing to the interdisciplinary nature of my research - much of it focusing on the chemical or biophysical aspects of phytochromes, I have routinely attended smaller conferences in the areas of photobiology, tetrapyrroles and biochemistry. The large size and overwhelming number of participants of ASPB meetings accounts for my infrequent attendance at annual meetings. Recognizing their value to career development of my students and post-doctoral fellows, I always have supported them to attend the ASPB annual meetings when they were ready to present a poster and/or talk on their research. When invited to an ASPB annual meeting, I invariably would send a student or postdoc so that they could network and advance their career by meeting with potential future mentors. I would also send eager students to speak and meet with the international community of scientists at smaller meetings as well since public speaking (and reading) has always been stressful to me.

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

The opportunities available to plant biology M.S. and Ph.D. degree recipients have changed drastically since I was looking for a job in the late 1970s. So any advice I could give has to be tempered with my limited knowledge of the ever changing landscape in plant science career opportunities. Clearly, one needs to be apprised of new disciplinary developments, so I would urge all

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early career plant biologists to join ASPB and engage in the annual meetings and outreach activities of the ASPB and other plant biology affiliated organizations. Identity your strengths, whether lab work, teaching/mentoring others, public speaking, environmental policy, nurturing plants, organizing data for presentation or web designing/ social media outreach (amongst many others) - and then strive to maximize your time spent working in those areas. In short, find what you enjoy the most and seek out opportunities which maximize your effort using these skills. It is also important to acknowledge your weaknesses and strive to find partners who complement your deficits. To me, self-validation has always been more motivating than expecting others to recognize your value. Maybe that will be the same with you - or maybe not. Be positive and treat others as you would wish to be treated. Most employers will recognize your passion and be eager to have you joint their team. Finally, when you find yourself not looking forward to most days, ask for help - we are all in this together. Your advisors and colleagues want you to succeed.

Academic Family Tree https://academictree.org/chemistry/tree.php?pid=81395