ASPB Pioneer Member

Virginia Walbot

I am a native Angeleno with ancestors in Southern California dating back to the early 1800s. I grew up outdoors with plenty of farm land and wild space (coastal marshes, scrub forests, salt ponds, beaches, and tide pools) nearby; all these natural spaces gradually disappeared, bascially gone by the time I was in high school as millions of people moved to LA. In addition to roaming free in the outdoors, from a young age I fixated on plants, requesting a rose bush for my 5th birthday rather than anything else. No matter where my family lived, I always found at least a small space for a garden.

In school, I was very good at sports and math – however, my impoverished schools used hand me down textbooks from the 1930s and 1940s. It's the 1960s: imagine learning 1940 geography with all the incorrect country names and biology without mention of DNA. By gathering discarded coke bottles at the beach and returning them for the rebate, I collected enough money to take the bus from the LA airport to the Natural History Museum and neighboring municipal rose garden almost every Saturday. I spent hours in the hall of minerals and learning the bones of the California megafauna –giant lions, sabertooth tigers, and other animals that had gone extinct in the recent past. Also, to learn more math, I got a card to the downtown Los Angeles main library and checked out math books. Through a lucky meeting at the LA airport and a conversation about linear algebra, I got a recommendation from a Cal Tech professor that admitted me to college math classes at the Jesuit Marymount College. Inspired by what seemed an unlimited chance to learn, I began researching college scholarships and entering math contests, because my family could not afford to pay for further education. Using my earnings and a miscellaneous collection of scholarships (thank you Elks, Chamber of Commerce, State of California and others), I chose Stanford over MIT for college and could pay for it.

College was super challenging as I had very poor study habits and basically no background in the humanities. I remember my first history lecture – it was about Mesopotamia – I couldn't spell it, I didn't understand any of the parallels drawn to other ancient cultures, and I didn't understand why we were studying it. I had classmates who had studied Greek and Latin in private schools who already seemed to know everything. I felt really lost and disadvantaged in the mission to get a liberal education. This changed when I started learning Italian and spent six months in Florence Italy at the Stanford campus there. With proficiency in a new language and the Renaissance context provided by the city, I learned to love art history and the cultures that built Italy, an appreciation that extends very widely now.

Although majoring in math was the original plan, after taking introductory biology courses I decided math was a hobby. I was entranced by the the alternation of generations and specification of a germ line in plants, and I began helping out at the herbarium and interacting with Professor Peter Raven and his colleagues. It was an exciting environment as Raven and Paul Ehrlich were developing the theory of co-evolution of plants and insects. Peter became my honors project sponsor, and thanks to his effort and the generosity of Winslow Briggs, I was given an entire small greenhouse for a one year project determining the chemicals in eucalyptus leaves that leached into the ground and altered the germination of native plants compared to invasives such as wild mustard and oats. During my senior year, I participated in the graduate seminars on plant population biology and ecology: this inspired me to apply for graduate school, and later that year I was thrilled to be accepted to Yale.

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At Yale, I switched from the eco-evo track to the cell molecular track, because the last course I took at Stanford was molecular biology and the concepts of mRNA and tRNA were compelling. After a few disastrous rotations at the Medical School in labs trying to do molecular biology, I gravitated to Ian Sussex' plant anatomy and development lab, reasoning that it's better to love the organism and implement new techniques than to use the techniques on questions I was not passionate about. The intellectual atmosphere in the Biology Department, and the Sussex lab in particular, was fantastic for me: there was a great emphasis on analyzing a problem before starting work and on collecting "descriptive" information to better understand the organism before designing experiments. The apex moment was a definitive experiment to test your best hypothesis. This mantra of think first, describe the system, and then do a beautiful and definitive experiment became the goal in all my work. My NSF fellowship-supported thesis concerned the development of bean embryos – charting their ontogeny and measuring aspects of RNA metabolism from inception through early stages of germination. I built a developmental timeline based on macro characteristics that allowed comparison among genotypes and acquisition of correctly staged material for biochemistry. A six week NSF-sponsored course at UC-San Diego, led by Maarten Chrispeels, in molecular techniques applied to developmental problems provided new methods to study bean embryos. I established that during germination tRNAs acquire post-transcriptional modifications required for function and that stored mRNAs were utilized to program early germination.

Anxious to focus on individual proteins and their mRNAs, I used my NIH postdoctoral fellowship in Leon Dure's lab at the University of Georgia. We monitored enzyme activity to gauge the timing and requirements for the synthesis of specific mRNAs involved in cotton seed germination, establishing that stored mRNAs in seeds lacked polyadenylation and that this post-transcriptional modification was added during imbibition, permitting translation. As a side project, I also analyzed the structure of the cotton genome, publishing the first such analysis of a plant genome. The most important activity was interacting with young faculty recruited by former Yalie Norman Giles to the new Genetics Department; collectively these faculty had all the tools for gene cloning, and UGA was pionoeering recombinant DNA techniques well before most institutions. We worked together, often at night, purifying restriction enzymes and other needed reagents. I contributed by analyzing hybrid products by Model E ultracentrifugation (separation based on GC-content) and by electron microscopy. The diversity of plant biologists at UGA was also eye opening, as Yale had few faculty with such interests. I eagerly attended seminars and classes to learn more about plant physiology, plant breeding, disease, and agronomy.

During graduate school and my postdoctoral training I was very involved in women in science protests: basically, why train us but not employ us? Mary Clutter and I lobbied AAAS Council members to set aside $50,000 to establish the Office of Women's Studies, which has morphed over the years into all of the AAAS activities that advocate for inclusion and diversity in science. Mary Lake Polan and I established Women in Cell Biology at the 1971 conference in New Orleans – ever poor, I hitchiked there from New Haven – and then for the next four years, Mary Clutter and I published a monthly newsletter distributed to ~400 people listing job openings (contributed by students, postdocs, and staff who spied on their local faculty) and tips for applying and interviewing in an era before jobs were advertised. It's ironic that my own first job came from the "old boys network"—a phone call from Joe Varner, newly arrived at Washington University, inviting me to join him in revamping plant biology there. Numerous times research associate Mary Clutter, postdoc Mary Lake Polan, and I were told by "important" men that our careers were doomed because of our advocacy activities. This was not a deterrent. Indeed, Mary Clutter joined the NSF and rose to the Associate Directorship,

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fostering many new programs in training and career advancement and spearheading a plant genome research program. Her impact on young scientists and the plant biology community was profound. Mary Lake Polan obtained an M.D. at Yale, and she became the first woman chair of a Stanford medical department, where she served many years while conducting innovative research on the earliest stages of human embryo development.

I flourished at Washington University, importantly discovering the joys of working with corn and doing genetics. Although recombinant DNA techniques were developing rapidly, the complexity of the maize genome was daunting; consequently, our gene analysis work focused on the chloroplast and mitochondrial genomes as tractable, projects that continued through the 1980s. This is probably impossible now: I got my first NSF grant with only a hypothesis and no preliminary data. I tested whether the dimorphic C4 chloroplasts developed after a plastid genome inversion to change gene expression. The answer was no, but a paper in CELL resulted. While I was an Assistant Professor, discussions and manuscript preparation with Bob Goldberg were an important contribution: Bob is a meticulous editor, and I finally learned how to spell and how to write more fluidly. I also interacted often with Peter Raven, then head of the Missouri Botanical Garden; he is a great sounding board for new ideas and a consistent champion of "going for the gold" by challenging long-held assumptions. After six years in St. Louis, including close collaborations with Gerry Neuffer and Ed Coe at the University of Missouri, I was offered positions at UC-Berkeley and Stanford in 1980. I chose my old Department because of the excellent collegial relationships and access to an on-campus corn field established by George Beadle.

The 40 years at Stanford have been wonderful. My lab has worked on very interesting scientific problems, and in parallel we have been spurred to invent new techniques for our own work and hence have contributed to the impressive array of modern techniques. Using NIH support in the 1980s and 1990s, we pioneered electroporation of protoplasts for transient analysis of gene expression, built the initial firefly luciferase vectors, discovered the intron enhancement effect, and documented other key features of mRNAs that boost translation. Many of our vector backbones are still used today.

In 1978 I got a surprise phone call from Barbara McClintock, inviting me to visit her at Cold Spring Harbor. I spent a few weeks there, learning more about corn and about her theories and evidence concerning transposons over marathan 16 hour days of work and discussion in her lab. This visit, and a subsequent longer visit in 1982, were the foundation for starting work on the hyper-active *Mu* transposons of maize that had been identified by Don Robertson. Again, supported by the NIH with the goal of being able to clone maize genes efficiently, we had funding for 25 years. We followed *Mu* activity utilizing mutable *bronze2* anthocyanin alleles as markers: we discovered that *Mu* silencing involved DNA methylation, we cloned and sequenced the master element and demonstrated it encoded two genes, each with several isoforms, and that activity peaked in male reproductive tissues. We calculated how *Mu* activities were deployed across the life cycle; we quanitified the distinctive developmental regulation of *Mu* elements and the universe of allelic diversity expected from *Mu* excision compared to other maize transposons. Many of these studies were improved through conversations with Mike Freeling and his lab, and there was a friendly competition on many questions. We later proved biochemically and in transgenic corn that the transposase existed in multiple isoforms with distinct functions. I showed that quiescent *Mu* elements could be reactivated by UV-B treatment of pollen, and this led to a multi-year inquiry of the impact of this radiation on maize, supported by the EPA and USDA. Along the way we cloned the first *Mu*-tagged gene (our activity marker gene *Bronze2*), elucidated the final steps in anthocyanin biogenesis involving BZ2 protein protecting anthocyanin from oxidation and delivering it to an Multi-drug Resistant Protein tonoplast pump,

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documented *Bz2* expression during stress from alternative transcription start sites, and showed that highly divergent Petunia and maize glutathione *S*-transferases were interchangeable in the BZ2 role.

The advent of the NSF Plant Genome Research Program (PGRP) in 1998 was a golden opportunity to build better tools for maize genetics. I led a team of 10 collaborating labs from 1998 – 2003 in the Maize Gene Discovery Project, which contributed hundreds of thousands of maize ESTs from diverse tissue sources, sequenced *RescueMu*-tagged genes and *Mu* tagging stocks, 30,000 mutated lines evaluated for seed, seedling, and a subset for field phenotypes, the first corn microarrays, and numerous other tools that accelerated maize research. Stanford was a hub of early genomics era research, and our project benefitted from pre-commerical knowledge of new equipment. While other projects were using DNA sequencing gels to gather EST information, we knew that capillary sequencing was "almost" ready; by waiting eight months making libraries without performing any sequencing, we started with two of the first capillary machines built and within three months had more ESTs that the gel-based labs had generated in a year. Our rapid pace and high quality libraries boosted the maize EST collection to third highest, just behind mouse and human for many years. In this era, my lab also collaborated with Rod Wing's group in a PGRP project to sequence ~30,000 full length cDNAs to gain a better understanding of gene structure and to aid annotation of the in progress maize genome.

By the early 2000s it was clear that *Mu* regulation was actually a problem in anther differentiation: in the precursors to the germinal cells, *Mu* elements exicsed and re-inserted, while in somatic cells the primary activity was excision only. I decided that my undergraduate lectures in plant reproduction had raised questions that were still unanswered and that we needed those answers to understand the regulation of *Mu*. Where does the plant "germ line" come from – how do cells switch from mitosis only to a lineage that undergoes mitosis but is capable of meiosis later? Is there a special hypodermal cell (textbook model) that divides to make a somatic and a germinal daughter inside a flower? When is the germinal lineage established? How does it differ from the strictly somatic surrounding cells?

Spurred by these questions, I obtained several pre-meiotic action, male-sterile mutants for observation. More mutants were needed: to solve this, we started a collaboration with Zac Cande's lab in 2007 to organize and more deeply understand the male sterilty mutants of maize. Funded by the NSF PGRP program and gathering resources from the community, we classified the time of action of more than 500 mutants and found 18 new genes involved in pre-meiotic cell fate specification or differentiation. These, plus the classic five mutants known for this developmental period, have formed the basis of our research the past decade. Early microarray gene expression analysis of immature anthers from these mutants generated a lot of data, but not much initial insight until I noticed that at a particular stage, just before we could observe archesporial cells cytologically, one gene expression signature was characteristic of hypoxic root tips. Could hypoxia be an enviornmental trigger of specification? Using borrowed equipment and then gassing developing tassels *in situ* with oxygen or nitrogen and then chemicals altering ROS, we established that hypoxia triggers germinal specification from a field of similar meristem layer2-derived stem cells – there is no special hypodermal cell – fate is set dynamically in response to growth, with the interior most cells of incipient anther lobes becoming hypoxic as demonstrated by measuring oxygen levels. Within weeks, we had the data for a landmark Science paper. The low oxygen triggers activity of MSCA1 glutaredoxin, which we hypothesize results in the activation of a suite of pre-existing transcription factors. This results in synthesis and secretion of MULTIPLE ARCHESPORIAL CELLS1 (MAC1) protein, which is perceived by receptors on neighboring cells as a signal to differentiate as somatic primary parietal cells. As a consequence a column of archesporial

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(germinal) cells is established in each anther lobe, surrounded by somatic parietal cells that divide periclinally to make the bilayered subepidermal endothecium and secondary parietal layer. I wish those studying rice and Arabidopsis anther development, where many of the same gene types are critical for germinal specification are required, would test the role of hypoxia experimentally as closed buds are typical of angiosperm flowers and should be internally hypoxic. Refined analysis of our mutant collection by quantifying the pace and pattern of cell division within each tissue allowed us to focus on mutants affecting individual cell types. We even discovered a new anther cell type, the interendothecium. Although most of our proteomics, RNA transcriptomes, and small RNA analyses have utilized carefully staged whole anthers, we've also used laser microdissection to recover specific cell types.

In 2009, collaborating with Sundar's lab at UC-Davis and Vicki Vance of the University of South Carolina, we discovered a new class of small RNAs highly enriched in male reproductive tissue: 21-nt and 24-nt phasiRNAs. We're still mystified by their functions, but have enjoyed a very fruitful collaboration with Blake Meyers' group in tracking their spatiotemporal deployment during anther biogenesis. Funded by the PGRP since 2012, this collaboration has charted the appearance of phasiRNAs during plant evolution, several mechanisms of biogenesis and distribution in specific anther cell types, and the ability of the 24-nt phasiRNAs to promote *in cis* DNA methylation. Building a robust context of anther development, we were among the first to implement single cell RNA-seq, initially focused on archesporial, pollen mother cells, and meiocytes and now extending to all anther cell types. To overcome the limitations of protoplast isolation to recover individual cells, we recently invented a revolutionary new technique of whole tissue fixation followed by cell isolation that yields more cells for excellent coverage of an organ with better RNA quality and no requirement for special transgenic stocks.

On a short sabbatical in 2009, I decided to think about anther growth control. I was struck by photos of *Ustilago maydis* infections that convert anthers and other floral structures to tumors. How can a fungus reprogram a plant? What is a tumor? The role of the tumor is to support production of fungal teliospores, a process that takes about two weeks from infection to spore release. I became curious about the corn tissue requirements to make a tumor and how it might fit into what we know, and did not know, about anther growth. I got the FB1 and FB2 *U. maydis* strains from Flora Banuett, plus a few simple instructions on propagating the fungus and infecting maize, and spent six months testing the fungus on normal corn and on some of our male sterile mutants. I discovered that early acting mutants that arrested anther growth also failed to support tumor formation, but tumors developed normally on leaves. At this point I had a flash of insight moment: the fungal genome had been published and hundreds of likely effector genes identified and hypothesized to be deployed to cause tumors .... What I thought was, but of course, leaves and anthers use different genes and mechanisms to regulate growth, so I predict that the fungus must use different effectors to program tumors in discrete organs of the plant and that plant cells are responsible in part for programming fungal gene expression based on the inability of male sterile mutants to support tumors. I arranged to visit the heart of Ustilago genetics research in Regina Kahlman's lab in Marburg and presented my paltry data and new idea to a very skeptical audience. Nonetheless, postdoc Gunther Doehlmann agreed to visit the lab for three months to test the idea with us – using microarrays and proteomics, we demonstrated distinct *U. maydis* infection programs in seedling leaves, adult leaves, and anthers at three and nine days post-infection. We published in Science: many reviews were solicited, because it was hard to accept that no one had thought of this simple idea of organ specificity in pathogen attack before. In the subsequent decade, we've worked with the now independent

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Doehlemann lab to establish tissue and even cell type specificity in *U. maydis* action on corn. A current single cell sequencing project in my lab is exploring the impact on infected anther cell types and neighboring uninfected cells. We've also exploited Ustilago to understand more about maize anther development using a new tool, the Trojan Horse delivery system, to allow timed secretion of small maize proteins such as MAC1 by Ustilago to correct developmental defects or to perturb anther development.

I feel exceptionally lucky to have had a career in scientific research: it's been far more rewarding than I ever anticipated. I've interacted with brilliant people and have had a hand in training several generations of successful scientists, administrators, and biotech professionals. Although there have been occasional financial worries, the lab has been generously supported for my entire career. Five former lab members are in the National Academy of Sciences, and many more have made substantial contributions to their fields. This is a wonderful legacy. I've also enjoyed working with high school and college students every summer in the corn genetics fields. Working at our two acre farm on campus has fostered close friendships and scientific collaborations, and we've had a lot of fun at our weekly barbeques. For 30 years, the lab also had winter fields on Moloka'i or Kaua'i to speed up our genetic analysis and *Mu* tagging efforts; for seven years we conducted large screens for new mutants at Cal Poly-San Luis Obispo plus ten years using four acres at the Webb Ranch at Stanford. Altogether, we have grown more than two million corn plants, and we annually screened more than a billion aleurone cells for purple spots indicating Bronze2 activity. With a well-annotated corn genome and many new tools, now we can accomplish more with a handful of plants in much less time. But I don't regret the intense field work – every year observation is keener, and the outdoor work is a great way to stay healthy and active.

Science has never been so rewarding as it is today: we have more resources and new tools to ask ever more refined questions. There are still many surprises, and both the thrill of the chase and the satisfaction in being the first person in the world to know something motivate me every day. I also know that the mantra think, observe the organism closely, and design a great experiment has served me and lab members well over the years. The temptation to just collect data and hope that insight pops out is especially prevalent now as there are high throughput methods to generate vast datasets. Careful, analytical thinking and pressing for quantitative answers will, however, provide the foundation for much more robust solutions to our questions.