ASPB Pioneer Member

JUDY CALLIS

How did you spend your career?

Growing up, I did not have biology on my mind. In fact, I avoided biology in high school after seeing my neighbor spend many afternoons with an insect net chasing bugs for a required collection in the only biology course offered. When I started college, I thought I would be a Russian major, but I got bit by the biology "bug" after taking an introductory biology course that used the textbook, "*A Course in Biology*", by Baker and Allen. I still have that book and even today appreciate its focus on scientific logic and experimentation, rather than "facts". However, I am not sure I appreciated it at the time. I do remember sitting in class thinking, "when are we going to learn anything?", while the professor discussed hypothesis generation and testing! I will never forget the black box he drew on the chalkboard that remained for several lectures. I am forever grateful for that class, which got me started.

Much more so than any aspect of animal biology, I was always interested in how some plant species survived stresses, especially after frosty nights, while their immediate neighbors were completely dead. As a result, I sought out classes with plants as the subject matter and was fortunate that Virginia Walbot, a new Assistant Professor in the Department of Biology at Washington University, accepted me into her laboratory as an undergraduate researcher. Her lab was full of passionate, dedicated individuals and

she gave them freedom to flourish. Learning how to do science in that lab was a wonderful experience. Dr. Walbot gave me lots of autonomy, and I am not sure I embraced it as much as I should have. I am also grateful for the support and mentoring that her PhD students gave me, and I returned the favor by stocking the hall bookcase with discarded paperbacks from my bookstore part-time job.

After my AB degree, I worked for a few years as a lab technician, but then returned to school as an MS student at University of Illinois, Champaign-Urbana. My mentor, Dr. David Ho, was enthusiastic, knowledgeable about hormone signaling, and incredibly supportive. I learned ballroom dancing at the U of I, but more importantly, while studying barley aleurone layer a-amylase isozymes induced by gibberellic acid during germination with David's tutelage, I also learned more about asking questions and answering them with the right scientific approaches.

After completing my MS, I switched institutions and studied for a PhD at Stanford University, working again with Dr. Walbot, who had recently moved there from Washington University. Again, her lab was intellectually stimulating and busy, full of smart people excited about their work, and the post-docs were great mentors and friends. I must admit that I struggled a bit to find a viable dissertation project, but while doing so I had a wonderful side project in collaboration with Justin Roberts, using *in vivo* NMR of maize roots to understand metabolic changes during hypoxic stress. We utilized genetic lines of maize I obtained and grew at Stanford (yes, there is a corn field there) that expressed various levels of ADH1 activity to assess the enzyme's role in hypoxia. My main project, which was on maize ADH1 gene expression, evolved into a study of the role of introns in enhancing gene expression (more below).

As I thought about what to study after my PhD, I became interested in a post-translational mechanism that regulates phenotype, i.e., protein degradation. In plants, very little was known outside of work on storage protein degradation, and so I sought a post-doctoral position with Dr. Richard Vierstra at the University of Wisconsin-Madison to learn all I could about the ubiquitin system and its role in regulated proteolysis. This area has been the major focus of my laboratory in the Department of Molecular and Cellular Biology at the University of CA-Davis now for over thirty years. This campus has an abundance of all types of plant biologists, making it a wonderful research community, but being embedded in a department that studies many different organisms helped keep me abreast of research areas outside of plants.

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

My dissertation research was impactful. I had been trying to use cDNA templates to express ADH1 isoforms in cultured maize cells and was not having any luck. To mix things up, I tried a genomic ADH1 construct and hurrah! Robust expression! In parallel, a post-doc, Michael Fromm, who pioneered maize transformation by electroporation and particle gun bombardment, was looking for promoters that would express well in cultured cells. Work on genetic engineering of plants with transgenes was in its infancy, and Mike worked to optimize the ADH1 5' flanking region in hopes it would be useful as a promoter for transformation constructs. However, despite heroic efforts, that promoter had not worked well. Once we saw that the ADH1 genomic sequence, but not the cDNA sequence, was effective, we characterized the effect of introns on expression of various gene constructs. Inclusion of the first ADH1 intron in expression constructs boosted expression ~50-fold, and it did not act as a typical transcriptional enhancer. For enhancement of gene expression, the intron had to be in the transcribed region and located near the promoter. Even constructs with only the first ADH1 intron and other promoter sequences gave strong expression. The use of introns for enhancing gene expression facilitated transformation research in monocots, and the inclusion of an intron is now routinely used to maximize expression of transgenes for basic and applied research of agricultural crops.

During my postdoctoral years, the exciting discovery of the ubiquitin system as a regulatory mechanism emerged, and I spent the bulk of my time cloning and characterizing ubiquitin genes and the enzymes that catalyze ubiquitination. Efforts charactering ubiquitin components are still ongoing today in many labs. As an independent investigator at UC-Davis, I continued to study aspects of regulated protein degradation. I sought a model to study the specificity and regulation of protein degradation and chose the Aux/IAA protein family, because it is an outstanding example of both features. My first brave graduate students, Cathy Worley, Nathan Zenser and Jason Ramos, had to develop systems to characterize Aux/IAA degradation. A series of papers identified the Aux/IAA "degron" (a protein region required for degradation), demonstrated that auxin modulated the Aux/IAA degradation rate, and discovered that a protein, AXR1 (a modifier of a group of ubiquitin E3s), influences their degradation rate. Subsequently, students Kate Dreher and Jonathan Gilkerson made seminal contributions (among other results), with Kate showing diversity in the degradation rates among Aux/IAA family members and Jonathan demonstrating a lack of ubiquitin site specificity and the absence of a requirement for lysine residues in Aux/IAA degradation.

As the immense scope of the ubiquitin system became apparent (~4% of the predicted Arabidopsis proteome is dedicated to this pathway!), members of the lab studied its various components. PhD student Chih-Wen Sun focused on regulation of ubiquitin expression itself, which is encoded by a small gene family. Chih-Wen demonstrated the utility of some ubiquitin promoters as constitutive drivers of expression; these promoters are used in many labs today. Undergraduate Susan Norris demonstrated an Arabidopsis ubiquitin intron has a stimulating effect on gene expression, thereby extending my dissertation studies and showing a broader effect of introns on gene expression. We studied the RING-type ubiquitin E3 ligases that interact with ubiquitylation substrates and catalyze ubiquitin conjugation. Our characterization of this family and their interaction with E2s (mainly by post-docs Herborg Herksdottir and Sophia Stone and PhD students Edward Kraft, Mon Mandy Hsia, and Damian Guerra) has been foundational in understanding the evolution and substrate-specificity of this family. In collaboration with Mark Estelle's laboratory, Jose Laplaza, then Magnolia Bostick and Sara Hotton, discovered and characterized an essential role for the ubiquitin-like protein, RUB; its conjugation to the cullin family of proteins regulates the activity of a whole subfamily of ubiquitin E3 ligases that are especially important for controlling transcriptional regulation of multiple plant hormonal signaling pathways, including auxin, jasmonic acid, gibberellic acid, and strigolactones. Our plant work led the way for studies of the RUB-like proteins in yeasts and mammals.

Over the years, there were other notable contributions by many students, postdocs and technicians, with the assistance of enthusiastic and talented undergraduates, and I apologize to those not specifically mentioned here. Supervising research

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projects and mentoring students have been the true pleasures of my career.

When did you become a member of ASPP/ASPB?

I became a member of ASPB in 1979. I don't remember signing up, and I don't remember the exact reason, but maybe it was to reduce the cost of registration to attend the annual meeting in Ohio.

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

ASPB became the most important scientific community of my career. I attended my first annual meeting when I was a technician, before returning to graduate school. And, typically over the years that followed, I tried to attend as often as possible. I found these meetings inspiring and so educational. As I taught general plant biochemistry for years, the annual meeting provided me an opportunity to keep abreast of new knowledge and understanding outside my immediate field of research, but within the scope of the course I taught. Also, I got to know so many scientists at the meetings over the years and seeing them at subsequent meetings created friendships (and meal mates!). The ASPB annual meeting provided me with opportunities to present my work and that of members of my lab, and it provided an opportunity for feedback from others.

I wanted to give back to ASPB, hence the reason for joining the Legacy Society, serving as Publication Committee Member/Chair, Secretary, and President. I hope the Society will continue to help create a strong plant scientific community, promote plant research, provide scientific leadership, guide scientific integrity, enable research publication, and support the budding careers of young plant scientists.

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

I highly recommend following your passions and interests, while looking for the significance and broad impact of what you love to investigate. Among other things, you will have to justify your research to either federal granting agencies, administrators, research leaders, or investors, etc., so be ready! Create and live in an environment where ideas can flow without recrimination, and where all are welcomed into the enterprise. We need everyone!

I encourage early career scientists to try to attend the ASPB annual meeting as often as possible. You will generate friendships, identify possible collaborators, and keep abreast of recent advancements. I know it can be challenging going to a large meeting while knowing virtually no one there, but it's worth the initial awkwardness. Remember, many attendees have the same experience and would love to meet you! The annual meeting provides mentoring and career guidance as well as workshops on new techniques and funding opportunities. In addition to that, there are science talks on a broad range of topics. The ASPB meeting complements smaller, more discipline-specific meetings. These types of small meetings, while very valuable, typically offer no career guidance or mentoring, so the ASPB annual meeting should remain on your to-go list.

Plant Biology Family Tree: https://academictree.org/plantbio/tree.php?pid=251045