ASPB Pioneer Member

Mark Estelle

I was born in Calgary Alberta and grew up roaming the prairie hills behind my neighborhood with a bow and arrow, ostensibly hunting gophers (unsuccessful). When I was older, I had more interest in literature (starting with books about robots and later moving on to the classics) than in biology. When I look back, I see my career was shaped more by instances of tremendous good luck than anything else. For example, I was very fortunate to receive both my B.Sc. and Ph.D. in the Genetics Department at the University of Alberta. This was a small department that was organism neutral. The theme was to use genetic approaches to study any and all biological problems. We did not speak of zoology, microbiology, or botany. I worked on Drosophila with my beloved mentor, Ross Hodgetts, but the departmental weekly seminars spanned all areas from virology to population genetics. It was a fantastic education and definitely conditioned me to shun silos. Genetic analysis, genetic dissection of diverse processes, really made sense to me and I was hooked. My thesis research was on ecdysone regulation of transcription in Drosophila and as I prepared to write my thesis, I started to think about what to do next. This is probably a good time to describe my planning process. I didn't have one! When you don't have a planning process luck is very important. As I was mulling over my next

move, one of the Professors in the Department, a fellow named Chris Somerville, was planning a move to Michigan State University to establish a lab at the DOE Plant Research Lab. Perhaps I would like to join him as a postdoc? To work on plants. A couple of things to note here. First, Chris was offering a position to someone who had zero first author papers. And second, I had no interest in plants whatsoever. Never thought about them except that I did enjoy photographing trees, particularly in the winter. Even now, I don't feel any special affinity for plants. No doubt my appreciation for plants has increased tremendously over the years. Plants are truly fascinating, but I am sure that I would feel the same way about a different group of organisms if chance had sent my career in a different direction.

Fortunately, regardless of my lack of plant-love, I did recognize that Chris was offering me a tremendous opportunity, one that I would be foolish to decline. Besides, I had no other offers. I moved to East Lansing in 1983 and began what was a life-changing experience working in Chris's lab. He had demonstrated at U of Illinois that big questions in plant biology could be answered by applying genetic approaches using the small crucifer *Arabidopsis thaliana*. My timing was perfect. This was the beginning of the Arabidopsis "revolution" and as each new student or postdoc joined the Somerville group, they were encouraged to identify a big problem that might yield to a genetic approach. Remarkably, Chris supported studies of trichome development, fatty acid biosynthesis, starch metabolism, purine metabolism, flower development, and hormone signaling (ABA, ethylene, and auxin), and I am sure I am forgetting research topics. It was a very exciting time, fueled by Chris's creativity, enthusiasm, and encouragement.

After several false starts, Chris suggested I study auxin biology and perhaps do a screen for auxin-resistant mutants. At the time, our knowledge of auxin signaling was rudimentary. From the work of Sakis Theologis and Tom Guilfoyle, it was clear that auxin promotes rapid changes in gene expression, but how this happened was completely unknown. The auxin literature was quite dense and confusing, but I was not encumbered by this since as a newcomer I was largely unaware. I did the

continued on next page

MARK ESTELLE *continued*

screen and was excited to recover a number of very interesting looking mutants including *axr1*, *aux1,* and *axr2*. This was early days before the Arabidopsis genome project and map-based cloning. Nevertheless, at the time a mutant with a phenotype was gold. In fact, in 1986 a set of interesting mutants was enough to get a job as an Assistant Professor at Indiana University in Bloomington, IN, and that is where I set up my lab.

Moving to Bloomington was another instance of very good luck. The Biology Department had a model-organism orientation and was loaded with outstanding researchers using genetically tractable systems such as Drosophila, C. elegans, yeast, and others. I fit right in. By this time, Arabidopsis research was becoming very hot, and I had three outstanding Ph.D. students before I had a functioning lab. At this point, I would like to formally acknowledge all the tremendous students, postdocs, and technicians that I have been lucky enough to work with. I appreciate every one of them. I am not going to name names here for fear of missing someone.

Once the lab was up and running the focus was on the mutants I brought with me from MSU. What could the mutant phenotype tell us about the function of the affected gene, and most importantly what protein did the gene encode? Nothing about this approach was new. It had been employed for decades in various animal and microbial systems and to some extent maize. We started with the most severely affected mutant, called *axr1*. We had done enough phenotypic analysis to know that *AXR1* had a key role in auxin signaling, but of course we had no idea about the identity of the protein. At the time, a number of smart people in the growing Arabidopsis community were rapidly developing the tools to enable positional cloning, or "walking" to the gene. RFLP maps, cosmid and YAC libraries, and improved methods for generating transgenic plants were all developed during this period. Despite these advances, cloning based on map position was still a very laborious process, especially since this was before floral dip transformation. Generating transgenic plants still required months of tissue culture. Fortunately, my team was incredibly determined and finally in the summer of 1992 we succeeded. I learned about our success while on vacation near Winslow, AZ (cue Eagles song) when I received an urgent message, relayed by my girlfriend's mother, to call the lab. What was this incredibly important gene? Was it a protein kinase? Perhaps a receptor-like protein or a master transcription factor? Nope, it was a mysterious protein that resembled the N-terminal half the E1 ubiquitin activating enzyme (which was what exactlly?). We were the first to identify a gene based on mutant phenotype in Arabidopsis, but the sequence told us nothing about the activity of the protein or how it might function in auxin signaling. When friends and colleagues learned of our success they offered congratulations, but also condolences. Nevertheless, we were confident that AXR1 had an important role in auxin response because the mutant phenotype told us it did. Future events justified our confidence.

The next several chapters in this story were written rapidly over an exciting 5-year period. We discovered that a gene called *TIR1* was required for the auxin response and encoded a member of the recently discovered class of proteins called F-box proteins. We showed that TIR1 assembled with other proteins into a type of ubiquitin protein ligase called an SCF complex. We were also studying two mutants called *axr2* and *axr3.* Importantly, Ottoline Leyser's lab cloned *AXR3* and showed that it encoded a member of a previously identified family of very unstable transcriptional repressors called Aux/IAAs. Further, we collaborated with the Leyser group to show that auxin promoted an interaction between TIR1 and the Aux/IAAs, resulting in degradation of the repressor. Meanwhile, other groups had identified a family of transcription factors called ARFs that interacted with Aux/IAAs. All of these discoveries fit together in a very satisfying model in which auxin promoted the degradation of the Aux/IAAs, leading to transcriptional activation by the ARF proteins.

continued on next page

MARK ESTELLE *continued*

But what about AXR1? How does it fit in? This mystery was solved by a very brave graduate student who resolved to use the yeast *S. cerevisae* to understand AXR1. All eukaryotic organisms encode an AXR1 ortholog, including yeast. We found that deletion of the yeast gene (we called it *ENR2*) did not result in an obvious phenotype. However, when we tried to make double mutants between *enr2* and other genes that function in ubiquitin-mediated process, we observed clear synthetic lethality. Surprisingly, we also observed a change in the apparent size of a protein called Cdc53. This protein is a member of the cullin family of proteins, subunits in SCF type E3 ligases. The meaning of this remained a mystery until a chance discussion with colleague Judy Callis at a FASEB meeting devoted to the ubiquitin-proteosome system. Judy was working on a small ubiquitin-related protein called RUB1 (NEDD8 in humans). We had both just heard a talk by a yeast biologist named Erica Johnson who had shown that another ubiquitin-related protein called Smt3p/SUMO was activated by a dimeric E1 activating enzyme consisting of two proteins related to the N- and C-terminal halves of the ubiquitin activating enzyme. Recall that AXR1 (and ENR2) resemble the N-terminal half of this enzyme. In mid-beer gulp, Judy and I both realized that AXR1 must activate RUB1, resulting in its conjugation to Cdc53p. We both went back to our labs where our respective students quickly confirmed that this was the case. Further, we demonstrated that Arabidopsis CUl1 was also modified by RUB1 in an AXR1-dependent manner. Our results demonstrated that SCF complexes are regulated by conjugation of RUB/NEDD8 to the cullin subunit. This result has been extended to all cullin-based E3 ligases in all eukaryotes. One of the important lessons of this part of the story is that IN PERSON meetings are very important. Zoom meetings can't replace chance encounters outside the lecture halls or at the bar.

At this point we knew that auxin regulated gene transcription by promoting the degradation of the Aux/IAA repressors. But how was auxin perceived? What was the long sought-after receptor? Answering this question was the highlight of my career, in part because the answer was so interesting, but also because of the collaborations and other scientific exchanges that enabled the work. In yeast and animal systems, substrate recognition by an E3 ligase generally involves a stable modification such as phosphorylation. Because we assumed that the same would be true for SCF^{TIR1} and the Aux/IAAs, we initiated biochemical experiments to identify proteins that promote the interaction. That was the plan until a gifted postdoc in the lab demonstrated that the interaction between TIR1 and the Aux/IAA occurred in the absence of any other protein. All that was required was auxin. Meanwhile a talented postdoc in the Leyser lab was obtaining similar results. Both groups worked independently, but in close communication, to show that the interaction between TIR1 and the Aux/IAA protein required direct but reversible binding of auxin to TIR1. This was a completely surprising and novel discovery and for auxin biologists, incredibly exciting. We later refined our understanding of what constituted the auxin receptor by showing that auxin binding requires both TIR1 and the Aux/IAA protein. In fact, the two proteins are co-receptors. Meanwhile, we had to acknowledge that the phenotype of the *tir1* mutant was rather modest. However, genetic studies revealed that five other related F-box proteins named AFB1 through AFB5 were also auxin co-receptors, and the loss of all six resulted in early embyronic lethality. These proteins are definitely important.

The discovery that SCF^{TIR1} -substrate recognition was regulated by non-covalent binding of a small molecule generated quite a bit of interest from the rapidly growing ubiquitin field. I don't remember who contacted who first, but my collaboration with a structural biologist and closet plant biologist, Ning Zheng, was a highlight in my career. Our contribution to the collaboration with Ning was small, mostly cheerleading. With a lot of elbow grease Ning's lab was able to express TIR1 in insect cells, grow crystals and solve the structure. I didn't ask for frequent updates, thinking that it might be a challenging project. I was very

continued on next page

ASPB Pioneer Member

MARK ESTELLE *continued*

surprised to get an email out of the blue from Ning. "We have solved the structure and it is very cool!" (I am paraphrasing.) When I opened that attachment, my mind was blown. The structure was beautiful and clearly demonstrated how auxin was perceived. The hormone occupies an auxin binding pocket in TIR1 and contacts a key residue in the degron domain of the Aux/IAA protein. Ning coined the phrase "molecular glue" to describe the function of the hormone. Later work by Ning and collaborators John Browse, Gregg Howe, and, Sheng Yang He demonstrated that jasmonic acid perception by the COI1 JA receptor works in a similar way. Parenthetically, the characterization of auxin as a molecular glue launched a cottage industry in the pharmaceutical space devoted to the identification of molecular glues that might be used to treat human disease.

The Department of Biology at IU was a wonderful scientific home, but I have always been most comfortable in the western part of the continent. Growing up on the prairies with the Rocky Mountains to the West, I longed for a distant horizon. In southern Indiana, the horizon is never distant. Shortly after the TIR1 structure was published I moved my lab to UCSD. Our work in San Diego has continued to focus on aspects of auxin signaling. In addition to Arabidopsis, we have worked increasingly with another excellent genetic system, the moss *Physcomitrium patens.* Our studies with this species revealed that the auxin signaling pathway is conserved among all land plants. Because it is relatively easy to do gene knock-outs and knock-ins, as well as gene editing, we can address fundamental questions concerning the function of individual auxin signaling proteins, as well as the architecture of the auxin signaling network.

As an ASPB "Pioneer' I think I am expected to provide wise council to young scientists. Because my career has been guided by good luck rather than planning, I am hesitant to offer much in the way of advice. However here are a few, somewhat idiosyncratic thoughts. Be sure to pick an important, challenging, but not intractable problem to study. Be aware of competition but don't let it distort your work. It is better to be open and collaborative than secretive and distrustful. It's more fun and will benefit you in the long run. Don't worry too much about networking if it makes you uncomfortable. If you do good science and publish it, people will notice. When you make an exciting discovery, be sure to celebrate right away, because it will be old news in a month. Finally, be sure to remind yourself why you love science as often as possible. Your motivation will be specific to you. For me, the discovery of something previously unknown to human-kind provides a rush like no other.