ASPB Pioneer Member

Richard (Dick) Trelease

How did you spend your career?

While earning my BS degree in Biology at the University of Nevada, Reno in 1963, I realized my interest in conducting physiological research on plants. This stimulated me to stay and pursue a MS degree with Hugh Mozingo, who had just recently received a NASA grant to investigate the effects of low pressure and Marsian atmospheres on terrestrial plants. UNR Botany also acquired a new electron microscope (EM) to document ultrastructural effects on plants exposed to NASA's Mars' gas mixture. I jumped at the opportunity to work on this project for my MS research. To study plants already established within an extremely low-pressure environment, Hugh and I collected plants on a field trip to the top of Mt. Whitney (14,505 ft elevation). I discovered that chloroplast membranes were most notably affected. This research gave me invaluable, first-hand knowledge of plant cell ultrastructure, with one caveat. NASA informed us later that the Mars gas mixture used in my study was different according to their most recent data. Nevertheless, I had become an experienced electron microscopist with a reasonably solid knowledge of plant cell ultrastructure, ready to go on for my PhD degree.

In 1965, I got married and we headed for the University of Texas, Austin. Gordon Whaley,

who became my advisor, already was involved in several electron microscopic studies of plants. Two organelles in germinated corn seeds that I clarified ultrastructurally were oil bodies and microbodies. Spherosomes were postulated to pinch off from endoplasmic reticulum (ER), then differentiate into mature oil bodies. I provided convincing evidence that spherosomes were a non-entity, and not involved at all in the formation of oil bodies. Furthermore, my EM images revealed that each oil body was not bounded by a so-called unit membrane, but rather by a novel, single protein boundary layer. This is the accepted interpretation today. Plant cytosomes were reported by others to resemble microbodies in animal cells. I confirmed that these plant organelles typically possessed diagnostic crystal structures indicative of peroxisomes, thus making the term cytosomes obsolete. It was an exhilarating time to be among the plant biologists who were directly involved in clarifying correct/appropriate names for some of these new plant cell constituents.

In 1969, I was awarded a NIH Postdoctoral Fellowship to further elucidate structure/function of plant peroxisomes (microbodies) in Eldon Newcomb's laboratory at the University of Wisconsin, Madison (UW). His group had a reputation for publishing exquisite electron micrographs. Consequently, I expected that his physical laboratory would be special—it wasn't! In Eldon's designated lab, students were mounting dried, flaky plants onto herbarium sheets. I was directed to an inner doorway where Eldon's EM and photographic darkroom were located, next to his specimen prep room where his sectioning microtome was also located. Eldon shared his surprisingly modest EM facility with the department's herbarium taxonomist. My first lesson was that top-notch electron microscopy was accomplished by people with extraordinary FINESSE and technical, artistic skills. I wanted to produce high-quality EM products at least equivalent to their level; I believe I came close before leaving Madison.

By this time, two major plant microbodies had been functionally characterized and specifically named. Glyoxysomes converted lipid body oil into carbohydrates, whereas leaf peroxisomes participated in glycolate metabolism

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(photorespiration). After doing some collaborative EM studies with graduate students, I worked mostly with Wayne Becker, a new physiologist at UW, who had established a collaboration with Eldon's group. We embarked on a multi-pronged approach with cucumber seedlings, whereby cotyledons were exposed to various light-dark, or dark only, regimes. The results led to a novel concept related to the transition of glyoxysomes to peroxisomes. *To wit*, the transition does not involve the actual replacement of one population of microbodies with another. Rather, the transition occurs within existing organelles via a change in the enzyme complement within a single population of organelles. Although controversial, this became the accepted model.

This had a substantial influence on my career, because the model was a foundation for my ongoing research plans as an Assistant Professor at Arizona State University (ASU). Friends and colleges in Madison shared my excitement as they wished me luck in my new position in Tucson, Arizona. (At that time, many people thought there was only one university in AZ; they soon learned that was not true!).

When I arrived at ASU in 1971, few Botany Department graduate students were interested in studying plant peroxisomes. Therefore, I recruited three Zoology Department graduate students who wanted to integrate EM into their research with animals. Glyoxylate cycle enzymes were reported in free-living vinegar eels and in parasitic *Ascaris* worms. Surprisingly, no EM evidence for glyoxysomes was observed in either nematode. Biochemical data led to an incredibly unique conclusion, i.e., glyoxylate cycle enzymes were located exclusively in the mitochondria of both nematodes. A mitochondrial localization has not been reported for any other eukaryote to date. As these Zoology students completed their degrees, my research transitioned back to plants.

Collaborations with plant physiologists at the Western Cotton Research Lab in Phoenix were a major boost to my research. John Radin and I initiated investigations aimed at regulation of glyoxysomal/leaf peroxisomal enzyme activities in cotton cotyledons during both seed maturation and post-germinative growth. Jan Miernyk, a Ph.D. student who unfortunately passed away this year (2020), made numerous critical contributions that were invaluable for getting cotton physiology research started in my lab. He established and cared for potted cotton plants grown in an ASU glasshouse, so we no longer had to travel daily to the Cotton Lab to tag flowers and harvest developing seeds. We were able to conduct more comprehensive cell biological, biochemical, and physiological experiment on both maturing and germinated cotton oilseeds.

New results revealed that glyoxysomes actually form while acquiring select enzymes during seed maturation, and then they differentiate following germination into enlarged, pleomorphic organelles via posttranslational acquisition of additional enzymes. Significantly, these events were NOT consistent with the popular ER (endoplasmic reticulum)-vesiculation model. Glyoxysome number was perceived to increase ONLY following germination, as vesicles "pinch off" from segments of rough ER containing co-translationally acquired enzymes. We realistically supposed that ER was involved both in glyoxysome formation/enlargement by providing membrane segments or components thereof.

Lively controversies persisted for several years, and I was able to express my views in a 1984 Annual Review of Plant Physiology article entitled "Biogenesis of Glyoxysomes." In 1983, Tony Huang, Tom Moore, and I collaborated in writing a book entitled "Plant Peroxisomes," which was sponsored by the Monograph Committee of the ASPP. The pros and cons of these models were thoroughly examined. Even though our interpretations fundamentally were correct, it became evident later that I had a greatly oversimplistic view of the actual multiple, complex roles of ER in plant cells

Kent Chapman, a PhD student, was interested in lipid/membrane biochemistry, and Javier Corpas, a postdoc from Granada, Spain, was experienced in peroxisome membrane proteins/enzymes. Both contributed substantial

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new complementary knowledge to ER-peroxisome biosynthesis/function. An interesting sidelight was that Javier met his future wife, Hiromi, in Tempe, when she was visiting/working from her home country, Japan. They married in Granada, where they raised their family, and after 25 years I still stay in contact with them. The same is true of Kent's family; he's a professor in Denton, Texas.

About 1995, my research emphases changed significantly, due to interests in intracellular protein trafficking. Accordingly, suspension-cultured tobacco BY-2 cells became our main experimental system. My lab groups had to become more competent in applying recombinant DNA technology in conjunction with confocal immunofluorescence microscopy. Scott Bingham, manager of the ASU DNA Lab, assumed a major role in this regard, and served as a Supervisory Committee member for many my PhD students.

Rob Mullen, a postdoc from Canada, came to my lab genuinely interested in collaborating with others on deciphering peroxisome targeting signals (PTS) and elucidating the multiple new roles of ER in peroxisome biogenesis. Teamed with Cayle Lisenbee and Robb Flynn, both PhD students, they resolved numerous PTS variations in amino acid positions as well as sequences for both peroxisomal membrane and matrix enzymes.

A vast amount of ever-changing data that became available in the mid-2000s led us to interesting and often unexpected modifications of peroxisome biogenesis models. These included multiple roles for the RER, and a novel, pre-peroxisomal compartment dubbed "peroxisomal ER". Peroxins, a new family of Arabidopsis proteins involved variously in peroxisome biogenesis were selectively investigated by PhD students. A model that I and student Matt Lingard published in 2006 provided a comprehensive montage of peroxisomal assembly models/pathways for several different cell types/organisms. A model Rob Mullen and I presented in 2006 was named "ER semi-autonomous peroxisome maturation and replication." For perspective, soon after I began my plant biology career at ASU, we challenged the simple ER-vesiculation model. Now, the incredibly more elaborate, complex models (very briefly described above) involve multiple specialized (sub)compartments of smooth and rough ER, wherein a myriad of matrix and membrane protein targeting signals participate in biosynthesis of new mature peroxisomes via one or more sorting pathways. It has been a wonderful ride directing and interacting with graduate students and postdocs who obviously had the ideas and skills to do this research.

Since my retirement in 2007, I have been active in a different area of plant biology. Specifically, I participate regularly as a multipurpose volunteer at the Desert Botanical Garden in Phoenix, where I am regarded as a "Hardy Perennial." I teach plant biology classes, emphasizing a Desert Plant Adaptations theme, to other volunteers, and at discovery stations with props along garden trails, I present interesting topics such as cactus skeletons, saguaros, and agave food and drinks, etc., to visitors. For teaching my classes and providing staff with examples, I have photographed many of the desert plants in the garden, illustrating their significant parts (waxy leaves, spines, trichomes, etc.). Bottom line, I traded the cameras mounted on the electron and confocal microscopes for my hand-held 35 mm Canon with telephoto and macro lenses.

What do you consider your most important contributions to plant biology?

When asked this question, I usually think about my specific scientific achievements, then wonder which one(s) to highlight. But through it all, my time and interactions with students are the most important. This includes undergraduates that I taught in the year-long major's biology course where I included much more teaching of plant biology than only the two lectures on photosynthesis taught by other biologists. Obviously, this also includes my graduate students and postdocs, particularly when we were planning, dissecting, and kicking around ideas, techniques, etc. I truly enjoyed interacting daily with graduate students, guiding, and challenging them to become

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competent, caring, questioning plant biologists. As my reward, almost every one of them have gone on to teach, train, guide, and/or stimulate others in the wonders of plant biology.

When did you join ASPP and what role did it play in your career?

I joined in 1969, when I became a postdoc at UW. A main role was preparing myself and later my grad students and postdocs to attend and present their research in talks or (mostly) posters at the annual meetings. For me, the best meetings were at universities, which fostered more intimate interactions, although dormitory beds and the lack of air conditioning did become less attractive (I slept outside on the grass with others at one meeting!) Discussing poster results, new ideas, and future research goals with experts in the field were invaluable experiences. After meetings, we mounted all our posters in hallways outside my lab, with the aim of students eventually publishing their studies. Hearing/meeting symposium speakers were also major pluses of being an ASPP member.

What advice would you offer to young people considering a career in plant biology?

Choice of where you study is important. Universities with Botany departments or Agriculture Schools have diverse undergrad plant courses, providing broad views/experiences in lab classes. If you are beginning graduate school, selecting a university with an MS program may be desirable, versus committing to PhD program; consider selecting graduate course credits that will apply toward a PhD In graduate school, seek collaborations with fellow graduate students and professors. Financial considerations have become important, thus being a teaching assistant whereby you can teach botany/plant physiology labs can be fruitful and enlightening. Diverse life science seminars and plant-oriented local and national meetings can greatly help one make the best decision for research training.