ASPB Pioneer Member

Kenneth Keegstra

How did you spend your career and what do you consider to be your most important contributions to plant science?

My scientific story begins in high school, where I became fascinated with chemistry. In large part, this was because of an engaging teacher. My first two years of college were at a local community college in northern Michigan, where again I was influenced by outstanding chemistry instructors. After transferring to Hope College in Holland, Michigan, I performed undergraduate research with an excellent mentor, Jerry Mohrig, who later moved to Carleton College, where he continued to have a positive influence on his students. A senior biochemistry class at Hope convinced me to consider biochemistry in graduate school. The Chemistry Department at the University of Colorado had a biochemistry section, where I worked in the laboratory of Peter Albersheim, a charismatic and creative scientist who had a strong positive influence on my career. Together with other graduate students, we investigated the structure of polysaccharides derived from plant cell walls. First, we used mild chemical treatments of insoluble cell wall preparations or purified enzymes to get wall polymers or defined fragments derived from them. We then used newly developed carbohydrate analytical methods to determine the structure of the various polymers or fragments. Combining this structural information with knowledge about the properties of the polymers and how they were released from cell walls, we proposed a model explaining how these polysaccharides might interact to form a functional matrix in the primary wall of plant cells. Although models of plant cell wall structure have evolved over the years, our model has received significant attention and was useful for designing future experiments. After undergraduate studies that focused entirely on chemistry, my graduate studies introduced me to plant biology, an interest that grew to dominate my career in later years.

In 1971, as I began to consider possible postdoctoral projects, the war on cancer had begun. So, I decided to explore the differences in surface carbohydrates between normal and cancer cells in the laboratory of Phil Robbins at MIT. Our strategy was to use viral glycoproteins as probes to query the structure of complex carbohydrates added by host cells to viral proteins. Because funding was readily available for cancer research, the path of least resistance was to continue these studies when I accepted a faculty position in the Microbiology Department of a new medical school at the State University of New York at Stony Brook. During my time at SUNY-Stony Brook, our lab also began pursuing studies aimed at understanding the biological function of plant lectins, including wheat germ agglutinin.

It was not long before I realized that studying animal systems was not my real passion. In 1977, when the Botany Department at the University of Wisconsin-Madison offered me a faculty position, my return to plant science was complete. The transition back to research on plant systems followed a meandering path that included continuing our investigations into the biological role of plant lectins, a project that was implemented by Michael Mishkind. A brief foray to investigate glycoprotein metabolism in plants led to our studying chloroplast envelope membranes, which we found lacked glycoproteins. After studying several aspects of envelope membrane biochemistry, including galactolipid biosynthesis and metabolite transport, our research group focused on the import of chloroplast proteins synthesized in the cytosol, a topic that we pursued for more than 25

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years. Over those years, our group investigated many aspects of the transport of precursor proteins across the envelope membranes. This included investigating the role of the transit peptide portion of precursor proteins, the binding of precursors to envelope membranes, and the energetics of the binding and transport processes. Together with Peter Weisbeek, Sjef Smeekens, and others in the Weisbeek lab in the Netherlands, we described the pathway by which plastocyanin moves from its site of synthesis in the cytosol to the thylakoid lumen, crossing three biological membranes in the process. In later years, we turned our attention to identifying the envelope membrane proteins and molecular chaperones that mediate the transport process.

It was an exciting time to be engaged in the field of organelle biogenesis. The new technologies associated with recombinant DNA methods and protein expression systems allowed novel precursor proteins to be produced and tested for transport into isolated chloroplasts. The availability of molecular cloning techniques allowed identification and characterization of components of the transport apparatus. This work was carried out by many talented postdoctoral associates and graduate students. Those who worked on the protein import project in Madison, included Ken Cline, Tom Lubben, Steve Theg, Alan Friedman, Kathy Archer, Barry Bruce, John Froehlich, Jaen Andrews, Maggie Werner-Washburne, Tom Moore, Laura Olsen, Cynthia Bauerle, Jerry Marshall, Hsou-min Li, and Sharyn Perry. Following my move to Michigan State University (see below), this project was continued by John Froehlich, Sigrun Reumann, Jennifer Davila-Aponte, Mitsuru Akita, Kentaro Inoue, Lynda Fitzpatrick, Joanna Tripp, Pat Tranel, Eric Nielsen, and Diane Jackson-Constan.

A significant change in my career came in 1993, when I moved to Michigan State University to become Director of the MSU-Department of Energy Plant Research Laboratory. With the move to a new location came a gradual transition to a new area of research. Although the chloroplast studies continued for another decade, we also began a new project focused on the biosynthesis of plant cell wall polysaccharides, especially xyloglucan. This research topic was chosen deliberately. On one hand, it was an easy choice, because the topic was familiar to me and was a logical extension of my PhD work on the structure of plant cell wall polysaccharides. On the other hand, it was a stretch topic, because the synthesis of polysaccharides is difficult to study, and progress had been slow during the 1970s and 1980s. The difficulty lies in the fact that polysaccharide biosynthesis is fundamentally different from the biosynthesis of other biologically relevant polymers. The sequence of monomers within proteins or nucleic acids is determined by templates, as described by the Central Dogma. For example, the sequence of amino acids in a protein is determined by the sequence of bases in the RNA template. On the other hand, the sequence of monomers in a polysaccharide is determined by the specificity of the enzymes that assemble the polymer from sugar monomers. Because most polysaccharide synthases and glycosyltransferases are integral membrane proteins, it is difficult to purify and characterize them. But the advances in plant genetics and molecular biology allowed different approaches to be pursued in the 1990s and beyond. During the past two decades, our research group, along with many others, made significant progress identifying and characterizing the genes and proteins responsible for making plant cell wall polysaccharides. As mentioned above, our lab focused on the biosynthesis of xyloglucan, a major polysaccharide found in the walls of all higher plants. The studies were carried out by many talented postdoctoral associates, graduate students, and staff members; they include: Ahmed Faik, Amy deRocher, Tanya Wagner, Teruko Konishi, Aaron Liepman, Olivier Lerouxel, David Cavalier, Yan Wang, Barbara Reca, Robyn Perrin, Weiqing Zeng, Jon Davis, and Linda Danhof.

As our cell wall research program ramped up, the chloroplast projects ramped down. By the mid 2000s, our research program was completely focused on plant cell wall polysaccharide biosynthesis. About this time, I made plans to

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step down as Director of the Plant Research Lab, so I could focus on research and teaching for the final years of my career. I even had the fantasy that I would again do experimental work with my own hands. But my well-intentioned plans did not materialize. In 2006, the US Department of Energy issued a call for new bioenergy research centers that would focus on the science needed to generate biofuels and bioproducts from plant biomass. Several of us at MSU joined forces with colleagues from UW-Madison and proposed creation of the Great Lakes Bioenergy Research Center. Much to our surprise and delight, our proposal was funded. Thus, instead of slowing down, I stepped into the most demanding position of my career, serving as Scientific Director for a multi-disciplinary research center with about 400 students, faculty and staff spread over multiple institutions. The Center includes a diverse collection of scientists, ranging from agronomists to zoologists and everything in between, and it has biochemists, chemical engineers, microbiologists, and plant biologists. The scale of the processes being investigated range from molecular efforts to improve cell wall degrading enzymes to ecosystem modeling, with the goal of improving ecosystem services while growing biofuel crops. The Center is still operational, and as they applied for an 11th year of funding, I decided it was time to let someone else have the fun.

The decision to retire is a consequential career choice, but one that is not discussed much among scientists. My personal decision to retire was influenced by a 2004 essay published in FEBS Letters by Jeff Schatz, an excellent scientist who studied mitochondrial biogenesis and is someone I had met at scientific meetings. During his retirement, Jeff pursued several activities, including writing essays that provide insightful commentary on aspects of the scientific enterprise. Although his essay on retirement, entitled “Letting Go”, is longer than this biography, his arguments can be summarized with two main points. The first is that scientists have an obligation to get out of the way to make way for a new generation of young talent. Secondly, he offered several ideas on ways scientists can remain engaged in the scientific enterprise if they wish to do so. At the beginning of 2017, I retired at the age of 71.

I think it is best to let others judge what constitutes my most important scientific accomplishments. I will briefly list those that I found most satisfying. The first was the creation of a cell wall model that tried to integrate the observations we made regarding the connections among the various polymers that constitute the primary cell wall of plants. While we never posited the model represents reality, it was useful for interpreting experimental observations and designing experiments to test the model. Another favorite topic was trying to understand how proteins cross biological membranes. It was particularly intriguing to understand how a protein such as plastocyanin, which is functional in the chloroplast thylakoid membrane, crosses three biological membranes following its synthesis in the cytosol and arrival in the lumen of the thylakoid membrane. Because it is a metalloprotein, it also needs to assemble with a copper ion to form the functional holoprotein. The details of the various steps are still not fully understood. A third topic I enjoyed was trying to understand how a complex polysaccharide, such as xyloglucan, is synthesized. While many important details are still not fully explained, much progress has been made in identifying the genes and proteins responsible for its synthesis. I first encountered xyloglucan as a graduate student while studying its structure. Indeed, our cell wall model postulated that xyloglucan is an integral wall component that connects cellulose to other wall polymers. Toward the end of my career, our research group used reverse genetics to demonstrate that Arabidopsis plants can survive and grow almost normally with undetectable levels of xyloglucan. It is still not clear to me how this surprising observation can be explained.

What advice would you offer to someone contemplating a career in plant science?

In looking back on a career of more than 50 years- from publishing undergraduate chemistry research to publishing collaborative research

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during retirement- it is amazing to see the progress that has been made in the biological and biochemical sciences. I have been enriched by pursuing research on diverse topics. Thus, one bit of general advice for young plant scientists is to follow their interests, even if it means switching research topics from time to time. Performing biological research requires utilization of so many specialized technologies that it frequently must involve a team effort. So, another bit of advice is to learn to communicate and work effectively with other members of a team. Clear and effective communication, whether written or oral, is an essential part of the scientific enterprise and its importance cannot be overemphasized. Ultimately, science is a human endeavor. I was influenced by outstanding teachers and mentors. In addition, I was privileged to work with superb colleagues at every step along the way, and with excellent students and associates, both in my own laboratory and those of collaborators. Now that I am no longer operating a laboratory, I find great satisfaction in reading their publications and following their careers.