Gary Stacey

How did you spend your career and what are your major contributions to plant science?

I grew up as part of a working-class family in Dayton, Ohio. Like so many, my father and mother migrated North after WWII and got jobs in the automotive industry, which was rapidly expanding in the Midwest. I was a bit of a hooligan, especially during my early high school years. Getting involved in sports was probably my salvation, and I avoided any serious problems.

My recollection is that my first real accomplishment in biology was winning the local junior high biological science competition. As crazy as it sounds, I recall reading an article in Popular Science that said you could learn while you slept. So to prepare for this competition, I read the biology textbook into a compact reel-to-reel recorder and then turned it on under my bed as I slept. To my surprise and that of most of my teachers, I won the competition. In retrospect, I am sure it was due to reading the book cover-to-cover and not any subliminal learning that took place while I slept. Regardless, perhaps this was my first step toward a career in biology, as well as my willingness to try new approaches.

While growing up, my father was an avid fisherman, and we would sometimes walk streams. My dad said rather than fishing, I would spend more time turning over rocks, looking for snakes and other critters. I point this out, since if there is one thing that describes my aptitude for science, it is curiosity. Indeed, I think an early manifestation of curiosity is probably one of the best indicators for someone who might have a future in science.

When I was in high school, one of the most popular shows on television was Jacques Cousteau's program describing his adventures around the world on his ship the Calypso. This led me to scuba diving. I was president of my high school scuba club and became fascinated by marine biology, which led me to matriculate at the University of Miami in Coral Gables, FL. On the first day of class in freshman biology the instructor asked how many students were interested in marine biology. Roughly two thirds of the class raised their hand, perhaps further testament to the popularity of the Cousteau TV show. The instructor then pointed out there were more hands going up than there were working marine biologists in the entire country. This was a rather sobering realization when one is anticipating a marine biology career.

Although I enjoyed my time at the University of Miami, it was an expensive university for me and my family, and hence after one year I transferred to Bowling Green State University (BGSU) in Ohio, where in-state tuition was much more affordable. I digress to point out this was the time when the Vietnam war was raging. I was able to get a college deferment, but the rules were that if you declared yourself eligible for the draft (1A) for one year and weren't drafted, you wouldn't subsequently be drafted unless a major war was declared. Your draft status depended on a lottery number determined by your birthday, and mine was 123. (That I remember this number decades after the fact attests to the trauma of this period). Given that my draft board had only reached number 90 or so the year before, I wrote them declaring myself 1A. That year they drafted up to 115 or so; hence, I was quite worried for a while. However, my strategy worked and hence I no longer had the draft to worry about and could move on with my life.

I chose to transfer to BGSU primarily because I had a high school friend there, and he sent me the application. It was the only application I made and, hence, my only option. However, it turned out to be a lucky choice, since there was a professor there, Cynthia Groat, who had established an active marine biology program. So, ultimately, I did get to pursue some of my original goals, although land locked in Ohio. As part of this program, the class would make an annual pilgrimage to the Gulf Coast Research Laboratory in Ocean Springs, MS. There I was able to take part in an undergraduate summer research program and worked with Dr. Cook, a marine microbiologist. This seminal opportunity set me on track to become a microbiologist.

After graduation from BGSU, I started

to apply for graduate school with the intention of becoming a marine microbiologist. This led me to the University of Texas and the opportunity of working with someone at the Marine Institute in Port Aransas, TX. However, after spending the summer there, prior to starting graduate school, I decided that my first choice of an advisor was probably not the right one for me.

After completing the summer program in Port Aransas, I drove 20 hours straight to Dayton and married my girlfriend. My wife and I then traveled back to Austin to begin graduate school. I had a bit of a letdown during my first semester in Austin, since I was mainly taking classes with no laboratory to call home; we did rotations. I came close to quitting, at least I seriously considered it. However, after the first semester I joined the laboratory of Robert Tabita and got started doing research; this ended any thoughts of quitting. Bob was an expert on bacterial ribulose 1,5, bisphosphate carboxylase, but my first project was purifying acetyl CoA carboxylase. I can't remember why, but Bob had some idea as to why this would be a good project. At that time, you could not buy acetyl CoA, so I first had to learn how to synthesize it, and I ultimately succeeded in purifying the enzyme.

Bob developed a collaboration with Chase Van Baalen, an internationally recognized expert on cyanobacteria. Van Baalen's lab was in Port Aransas and, knowing of my interest in marine microbiology, Bob suggested I spend a summer there. Chase assigned me the task of isolating nitrogen fixing cyanobacteria from the blue green algal mats common along the Texas coast. I did several enrichments and succeeded in getting a few axenic strains in culture. One that I termed *Anabaena* CA (sample C, isolate A), was a filamentous, heterocystous cyanobacteria that grew much faster than anything described in the literature. Hence, my first scientific paper was a characterization of its growth characteristics and nutritional requirements (it was truly a marine strain, as there was a requirement for sodium). The paper shows I rotated the cultures at 78 rpm under lights: I jury-rigged an old record player so I could use it as a shaker for my experiments. The CA strain became the subject matter for my dissertation and, again, was a seminal moment since it started me on a career focused on biological nitrogen fixation.

A side story to this period is that there was significant interest in my CA strain from other laboratories working on cyanobacteria, but Bob, presumably due to competition, never sent it to anyone. Hence, I thought it was lost. I am grateful that my friend Himadri Pakrasi at Washington University spent the time and effort to find and recover it. He has used it for experiments and shown it can be transformed. The moral is one should always share, since over the long haul your work and that of the wider community will be better served.

Given the expertise of the Tabita lab, my dissertation was primarily the enzymology of glutamine synthetase from the CA strain. I received a good education in microbiology and learned to be a solid experimentalist under Bob's tutelage. I also had an outstanding PhD committee, with three National Academy of Science members, Esmond Snell, discoverer of several B vitamins, Jack Myers, well known for his work on photosynthesis, and David T. Gibson, known for his work on biodegradation. This experience convinced me I wanted to focus on biological nitrogen fixation for my postdoc, which at the time was a hot field.

The laboratory of Winston Brill at the University of Wisconsin was at the top of the nitrogen fixation field, specifically the genetics of nitrogen fixation. Hence, I badly wanted to join his laboratory. I sent him a letter with my CV and soon after received a rejection saying he didn't have room. I was disappointed but noticed he would be giving a lecture at the American Society of Microbiology regional meeting in Dallas. I wrote back and said I understood and asked if we could still meet at this branch meeting, and he agreed. I went to the meeting with a few others from the Tabita lab. There was a cocktail hour before a joint dinner after which Brill gave the plenary talk. Afterward, I looked for him to have a discussion. As the featured speaker, he was surrounded by admirers. Knowing he had rejected me, I assumed he wasn't all that interested in speaking to me, so I engaged in some serious comradery with my colleagues during the cocktail hour. Consequently, I was not in the best of shape when he sat down in front of me and said 'let's talk'. I don't recall much of what I said but a few weeks later Brill sent me a letter offering me a postdoctoral position in his laboratory.

My time in Madison was good, both science-wise and with the birth of my son. Those were productive years working on the genetics of nitrogen fixation in the bacterium, *Klebsiella pneumonia*, while also starting my

work on symbiotic nitrogen fixation in soybean. My primary reason for joining the Brill lab was to work on *K. pneumonia*, but at the time Winston only had funds for me to work on soybean. However, shortly after joining the lab I was awarded an NSF-funded postdoctoral fellowship, which allowed me the freedom to choose projects. By then I was making progress on the symbiosis project and, therefore, ended up working on both *K. pneumonia* and nodulation. My colleague, Dale Noel (now retired from Marquette University) and I isolated some of the first nitrogen fixation and nodulation mutants of *Bradyrhizobium japonicum*, the soybean symbiont. Perhaps the most notable accomplishment using *K. pneumonia* was a paper in the Journal of Biological Chemistry, where we were able, using all purified proteins, to reconstitute the electron transport chain *in vitro* to support nitrogenase activity. To this day, I am not aware this has been done for any other system. This work was done with Dr. Vinod Shah, who is undoubtedly the best biochemist I ever had the pleasure of working with. Nitrogenase biochemistry requires specialized skills in anaerobic biochemistry, and it was a great opportunity to work in the Brill laboratory where so many great discoveries were being made. While Tabita trained me to be a bench scientist, it was really my time in Brill's lab where I learned the skills of how to manage a lab, motivate people, etc.

After about a year and a half in Brill's lab, I started getting nervous about my future, since some of the more senior postdocs were having trouble finding positions in academia. Hence, I thought it best to start applying. I quickly got my first interviews and not knowing what the future might hold, I took a position at the University of Tennessee (UTK) in the Microbiology Department. Luckily for me and somewhat unusual, Art Brown, who was chair of that department, agreed to allow me to delay joining the department one year, which allowed me to finish some of my projects, get more papers published, and better prepare myself to run my own laboratory.

At that point, my skills were in the areas of microbiology, genetics, and biochemistry. However, molecular biology was the currency of the day, and for that I was ill prepared. Luckily, when I joined the UTK faculty there was a full professor occupying my lab space. Fortunately, Karl Sirotkin, also a new assistant professor, offered me the use of part of this laboratory. Karl worked on Drosophila and had done postdoctoral research with Eric Davidson at Caltech. It was during this period that I was able to pick up molecular biology skills. I had sent a grant proposal to NSF prior to joining UTK, and it was funded within a few months of moving to Knoxville. I am proud to say some 40+ years later my research has been continually federally funded since then.

When I started my independent research, I focused on the *B. japonicum* mutants generated in the Brill laboratory. In addition to federal funding, I was also able to obtain a large industrial grant from Allied Corporation, which I believe at time was the second largest industrial grant awarded to UTK. Shortly thereafter, Allied brought Robert Goldberg (UCLA) and Arun Chatterjee (Univ. of Missouri) to UTK to review my program, which had really only just begun. This turned out to be another seminal moment in my career, since the advice that these two experienced scientists offered and which I subsequently adopted made a huge difference in the lab's future success. Bob has remained a friend throughout the years, and I still value his advice, while Arun subsequently became a colleague when I moved to the University of Missouri.

The Tennessee years largely focused on the genetics of *B. japonicum*. Highlights included cloning the nodulation genes of this bacterium, elucidating the regulation of nod genes, and isolating and chemically characterizing the lipo-chitin nodulation factor made by the bacterium. This latter work was done in collaboration with Russell Carlson (Univ. of Georgia) and Herman Spaink (Leiden University). In part, this was made possible by me being named the Van Der Klaauw Visiting Professor at Leiden University, which allowed me to stay in Leiden for a few months. We patented the Nod factor, which was subsequently licensed by industry and, along with patents from France, was the basis for Optimize™, a supplement for soybean rhizobial inoculants sold to farmers. To this day, Optimize™ is one of the few practical outcomes to come from basic research on symbiotic nitrogen fixation.

As my research program developed, it became obvious that future progress on understanding the soybean-rhizobium symbiosis required I transition my efforts away from the bacterium and focus more on the plant. Therefore, I arranged for a sabbatical in the laboratory of Josef Schell (Max Planck Plant Breeding Laboratory in Cologne, Germany) that was funded by a fellowship from the

von Humboldt Society. In the Schell laboratory, I worked with Frans de Bruijn and others, including Michael John, Jurgen Schmidt and Robert Masterson. The one year in Germany led to several nice papers and set my laboratory on the path of plant biology.

Upon returning to Tennessee, we focused on understanding the plant response to *B. japonicum* inoculation, a topic we still pursue today. After identification of the Nod factor, the next big question was the identity of the plant receptor. We initially tried a biochemical approach, reasoning that the Nod factor, a modified chitin molecule, might have a receptor similar to that which recognizes chitin. While the nod factor induces nodule formation, chitin elicits a strong immune response to invading fungal pathogens. The leading lab at the time in the study of chitin recognition in rice was that of Naoto Shibuya (Japan), and we initiated a collaboration. One of my Ph.D. students (Brad Day) spent a summer in the Shibuya laboratory. Ultimately, a genetic approach (using plant mutants) led to the identification of the Nod factor receptor and our efforts were in vain. However, this work did generate interest in plant chitin recognition, and we were subsequently able to identify the chitin receptor, CERK1, in Arabidopsis at roughly the same time as the Shibuya laboratory. We now know that CERK1 and the Nod factor receptor (NFR1) are structurally very similar (both LysM-RLKs) and, indeed, CERK1 can play both an immunity and symbiotic role in some plants. We went on to describe a second receptor (LYK5) and characterize many of the downstream signaling events involved in chitin recognition. This work also sent us into studies of plant pathology, another theme we continue.

The foray into chitin signaling led the lab to develop a focus on *Arabidopsis thaliana*, which turned out fortuitous, since it trained us in a variety of skills that we subsequently have been able to apply to soybean. In Arabidopsis, we dabbled in peptide transport, cloning the first peptide transporter of the PTR family and, subsequently, oligopeptide transporters (OPT).

The evolution of the laboratory's research into one primarily focused on plant molecular biology was not a particularly good fit for a microbiology department. Hence, when I was offered an endowed professorship in the Department of Plant Microbiology and Pathology at the University of Missouri (MU), it was not a difficult decision to move to Columbia. My career here has flourished, and the lab continues to explore various questions related to symbiosis and plant pathology.

Highlights of these years include co-leading an effort to sequence the soybean genome, resulting in a highly cited 2010 paper in Nature. Before single cell transcript sequencing became possible, we saw the need for a single cell system to study nodulation. Like many plant responses, only a few cells within roots initially respond to rhizobial infection and, hence, considerable signal dilution occurs when an entire root is extracted (e.g., for RNAseq). Therefore, we adopted some old methods to isolate root hair cells (the initial site of rhizobial infection) and in a series of papers used this approach to characterize the very early steps in rhizobial infection. Among the discoveries from this work was realization that the plant immune response is quickly triggered upon rhizobium inoculation and is then actively suppressed. We later showed the Nod factor is part of the suppressive mechanism, identifying a novel and unexpected role for what was previously considered a morphogenic signal. The dogma was that the Nod factor was unique to the rhizobial and mycorrhizal symbiosis. We now know that most filamentous fungi make similar molecules. It was assumed the reason why non-legumes do not nodulate is because they cannot recognize a Nod factor, but in other work we were able to show that corn does indeed recognize a Nod factor, and this stimulates root growth. Hence, the lack of nodulation by non-legumes is not due to their inability to recognize a Nod factor, but more likely is the inability to couple this recognition to the morphogenic program that allows for infection and nodule formation. This is relevant to ongoing efforts to engineer symbiotic nitrogen fixation into nonlegumes.

Early on, one protein our mutant studies implicated in nodulation was apyrase, which degrades ATP. The specific apyrase involved is an ectoapyrase, meaning the catalytic site is outside the plasma membrane. This implies extracellular ATP (eATP) must exist in plants, something we later visualized using luciferase. As it so happens, Gary Weisman, a member of the Biochemistry faculty at MU, is an expert in purinergic signaling (response to eATP) in mammalian systems and, hence, I arranged to meet with him. He described the history of purinergic signaling in animals and explained how the field was strongly criticized early on. After all, it seems crazy to use such an important molecule, the energy currency of the cell, as an extracellular signal. Indeed,

acceptance of the concept of eATP only gained traction after the first animal receptors were identified. It became clear to me that our first goal should be the isolation of a plant eATP receptor. However, there appeared to be no obvious homologues of animal eATP receptors in plants. Learning a lesson from our failed attempt to isolate the Nod factor receptor, we took a genetic approach and succeeded in identifying P2K1, a lectin receptor kinase, as the first plant eATP receptor. We have gone on to identify a second receptor P2K2 and demonstrated its role for purinergic signaling in plant growth, cell death and plant immunity. Purinergic signaling remains a major topic of research in the laboratory. What we are finding is that purinergic signaling underlies most plant stress (both biotic and abiotic) responses. Hence, if you are working on plant stress then you are likely also working, perhaps unknowingly, on purinergic signaling.

When did you become a member of ASPB?

I don't recall the exact year, but it was some time ago.

How did the Society impact your career and what motivated you to become a Founding Member of the Legacy Society?

Perhaps like many people, my first interactions with the Society were through its journals, either as a place to publish our work or through serving on the editorial board of Plant Physiology. I was then appointed to the ASPB Public Affairs Committee, serving as chair from 2006-2011. I was also a member of the Executive Committee from 2008-2011. Most notably during this period, I initiated and co-organized a meeting sponsored by ASPB, the Howard Hughes Foundation, and NSF to develop a decadal strategy for plant biology, which I believe was the first such effort.

ASPB has impacted my career in a number of ways, including providing a high-quality venue to publish our work. The annual meetings are always a great place to recruit students and postdocs, with stimulating collegial interactions and opportunities to share our science with the greater plant community. My years on the Executive Committee, especially the Public Policy Committee provided an invaluable education in public policy, the workings of Washington, DC and, perhaps most importantly, the confidence to try and do bigger things, not just in science but also in scientific policy and public outreach. The decadal strategy meeting mentioned above is one such example. The one thing I am most proud of is that I was co-founder of the Missouri Energy Initiative (MEI; www.moenergy.org), a non-profit corporation focused on energy policy within Missouri. This is a unique organization that brings together disparate groups from academia and industry, environmentalists and energy producers, to have productive discussions about energy. The public policy experience I gained through ASPB provided me with the confidence to undertake the initial development of MEI, and although I am no longer directly involved, MEI continues today.

As your career develops, you feel a responsibility to give back and provide the kind of support and encouragement to others that was so instrumental in the development of your own career and life. Given the education and support I received through involvement with ASPB and the role of this Society as a major spokesperson for plant biology in the USA and beyond, it was not a difficult decision when I was asked to contribute to the Legacy Society. I am certainly proud to be among the Founding Members of this group.

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

- First and foremost, follow your passion, your curiosity.

- I always ask…what is the most important question…what to work on? Or what not to work on? Perhaps the most important is the latter. As a curious scientist, you will have thousands of ideas, but the hands and resources to only work on a few. Hence, it is important to know which questions to throw away and which few to focus on. Important problems yield important results.

- Your research environment will influence your success, as well as your scientific evolution. Hence, strive to work in a rich, creative, and supportive environment.

- You are only as good as the people you work with. It is better to have no one in your lab than folks who are not contributing to the advancement of your science. I always encourage beginning assistant professors to maximize their own productivity. In the beginning,

you are clearly the best scientist in your lab, so capitalize on this until you establish your program.

- Soft skills are important. Work on developing relationships and collaborations. Don't be afraid to ask for help, especially as you enter new areas of research. You will be surprised at how generous colleagues are when you ask for help or advice.
- Modern plant science is big data driven; hence, plant biologists of the future need to be equally comfortable with computation/ data analysis, as well as data generation.

Academic Tree:
https://academictree.org/cellbio/tree.php?pid=784632