Sarah Grant

How did your career get started?

I was an undergraduate at the University of Guelph in Canada, which was built upon the already well-established Ontario Agricultural College. There, I learned to appreciate some of the economic and ecological challenges of agriculture. I read about the green revolution with its promises and its controversies. At the time, the late 1970s, genetic engineering and DNA sequencing were new fields. I was excited about the possibility of using genetic engineering to produce plant varieties with increased yield that could be grown in environmentally friendly ways for sustainable agriculture.

To start, I needed to learn about gene technology. In the late 1970's, Stanford University was already a hotbed for molecular biology, and I had read gene cloning papers by Stanford professors Paul Berg and Stanley Cohen in my senior classes. Our undergraduate Genetics Club ran across a short movie called "Protein Synthesis: An Epic on the Cellular Level" which featured Paul Berg as a straightlaced scientific expert explaining the current molecular understanding of protein synthesis by ribosomes. The process was then presented as a wildly whimsical dance by a collection of students and professional dancers and punctuated with lines from Lewis Carroll's Jabberwocky (https://www.youtube.com/watch?v=u9dhO0iCLww). I said to myself, I want to study with people like that and I decided that I had nothing to lose in applying to Stanford for grad school. Although he was not part of the movie, I wrote to Stan Cohen of the Stanford Genetics department explaining my hope to join his lab as a graduate student and learn to use gene technology in agriculture. I was thrilled when Stan wrote me back and encouraged me to complete the application process. In 1981, I joined Stan's lab as a doctoral student working on bacterial gene regulation where I learned how to splice DNA fragments together.

At that time, several labs were close to making transgenic tobacco using Agrobacterium transformation. At Stanford in 1982, Jeff Schell presented the achievements of his group. I decided that when I finished my Ph.D. I should try to head for the Max Planck Institute for Plant Breeding in Cologne (MPIZ), where Schell and others were using molecular biology to open the new field of plant molecular biology. Stan Cohen and Virginia Walbot introduced me to another of the pioneering scientists at the MPIZ, Heinz Saedler, when he came to speak at Stanford. I had read about Heinz Saedler's work in Virginia Walbot's graduate student class. He was studying transposons in snapdragon and maize and using transposons to identify and clone plant genes. The lab seemed a good fit. Heinz also had a history of having senior women scientists in his group, which made the group a welcoming place to do a postdoc.

Early in my time at Stanford, I met a student researcher in the lab of Len and Lee Herzenberg, Jeff Dangl. I was interested in genetic anomalies and Len's lab was doing work on intergenic recombination in the genes for immunoglobulin, so I did a rotation in the lab before I started in Stan's lab. Len figured I could help Jeff with purification of some of the many antibodies on his list, and Jeff and I became more than friends. We started to plan to do postdocs in the same town. Part way through his graduate studies, Jeff discovered the literature of plant-pathogen interactions. He realized that the genetics of disease resistance described in classical experiments suggested that plants produce immune receptors to recognize specific pathogen proteins. Klaus Hahlbrock, a leader in the field of plant biochemistry of pathogen responses, had recently joined the MPIZ in Cologne. He was happy to have Jeff, with his background in mammalian immunology, join his group as a postdoc. So, in 1986, off to Germany Jeff and I went, and our careers in plant biology began!

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How did you spend your career?

The MPIZ was an incredible place at the time, filled with ambitious young students and postdocs who have since made important contributions to plant science. We had every opportunity to learn about plant biology, genetics, and applied gene technology, and we had access to the most advanced resources available. I worked in the group of Alfons Gierl in the Saedler lab. Collaborating with maize geneticist Peter Peterson from Iowa State University, we analyzed the Spm transposon system first described by Barbara McClintock. I was part of a team of talented and dedicated students and postdocs. We identified the Spm proteins that are needed for transposition and affect expression of genes neighboring a transposable element, and we identified their DNA binding targets.

After Jeff and I had been postdocs at the MPIZ for four years, Jeff was offered an incredible research position in a new institute being built on the MPIZ research campus. The Max Delbruck institute was created as six independent research groups led by young scientists, including Jeff, working on cutting edge projects, some with plants and some with animals. Each group had five years of funding from the Max Planck Society and the Germany Ministry of Engineering and Science (BMFT). At the same time, Heinz, with Alfons' help, offered me the chance to apply for a five-year fellowship from the BMFT to lead a research team within Heinz's department to study the genetics of sex determination in dioecious plants. It was a fabulous opportunity. I had the benefit of top-notch mentorship from Heinz and the senior group leaders in the MPIZ, funding without having to write extra grant applications, access to cutting edge technology, and the chance to supervise ambitious and talented young scientists attracted to come to the MPIZ (See Plant Biology Tree, Sarah Grant: https://academictree.org/microbiology/tree.php?pid=392551). I was especially indebted to the Saedler group leaders, Zsuzsanna Schwarz-Sommer and Hans Sommer, for mentoring me as I learned to lead a research group. Heinz suggested we use Silene latifolia as our model dioecious plant because its sex-chromosomes had been relatively well characterized by cytogeneticists. As in mammals, females have two X chromosomes and males have one X and a Y chromosome which does not recombine with the X chromosome in meiosis. To find genes involved in sex determination, we X-rayed pollen grains, fertilized females, and selected the progeny that lost male characteristics. I destroyed two extremely expensive cathode X-ray tubes doing those mutation experiments. Finally, we generated a collection of over 50 mutants with either hermaphrodite or asexual flowers. We started an invaluable collaboration with the group of Boris Vyskot in Brno, Czech Republic, who showed that many of our mutants had Y chromosomes with visible deletions. At this point, the five years were up. Jeff was offered a faculty position at the University of North Carolina in Chapel Hill Biology Department, and I was offered a research faculty position. So, in 1995, off to North Carolina Jeff and I went to start our faculty careers.

One of the first things we did was hire a lab manager to help organize the lab space Jeff and I would share. We were lucky enough to find a dedicated scientist with a Masters degree in plant pathology, Terry Law. Terry has been the backbone of our group since then, doing essential research as well as holding the lab together on a personal as well as a material level. I continued the sex determination project along with my former graduate student from Cologne, Sabine Lebel-Hardenack, postdoc Richard Moore, and visiting scientist Sachihiro Matsunaga from Tokyo University. Ultimately, with mapping techniques from geneticist Beth Hauser of Duke University, and cytogenetics expertise from the Vyskot lab in Brno, we mapped the locations of many of the mutations using PCR markers. Male fertility mutations were associated with deletions on one arm of the Y chromosome, but mutations that affected female fertility were associated with deletions on the other arm. Evolutionary theoreticians had proposed that sex chromosomes would carry genes for female fertility and other genes for male fertility. The male Y chromosome would carry alleles that repress female fertility and enhance male fertility. If these two types of genes were on opposite sides of the Y chromosome, recombination with the part-

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ner sex chromosome would have to be repressed. Otherwise, recombination could lead to production of asexual flowers. Our mapping supported the theory that the male and female fertility genes would have to be on opposite arms of the non-recombining chromosome. The logical next step would be to molecularly characterize the sequences of the Y chromosome and to identify the relevant genes. Unfortunately, moving to cloning the interesting genes did not seem technically realistic because S. latifolia has a huge genome, the Y chromosome DNA is composed of mostly repetitive sequences, and we were not able to make transgenic S. latifolia plants. So, in 2003, I reevaluated my research program.

I decided to change course and study the plant immune system with Jeff's research group. Once again, I had the good fortune to become part of a large team of dedicated and talented peer scientists working toward a common goal. Terry and I joined a project led by postdoc Jeff Chang (now at Oregon State University), using bacterial genomics and a clever Fluorescence- Activated-Cell-Sorter-based assay to identify secreted virulence proteins known as type III effectors. The Guard Hypothesis of plant defense, articulated by Jonathan Jones of the Sainsbury Lab and Jeff in 2001, predicted that secreted virulence factors from diverse pathogens would enter host cells and interact with a relatively small group of plant proteins. These proteins would be important players in a common (basal) immune response that protects plants against most pathogens. To test this idea, we needed to identify virulence proteins with diverse mechanisms of action from a variety of pathogens and define their host targets. Many of the members of the lab were already studying plant pathogen type III effector proteins that elicit immune responses in Arabidopsis through recognition by NLR immune receptors. These effectors could be used as tools to identify the proteins of the plant basal immune response. In collaboration with Joe Ecker of the Salk Institute, Marc Vidal of Harvard, and Pascal Falter-Braun, now at Ludwig Maximillian University, Munich, we generated a collection of cloned type III effector genes for high-throughput yeast two-hybrid screens of the Arabidopsis genome for proteins that bind to pathogen virulence proteins. The results confirmed the predictions of the guard hypothesis that multiple pathogen virulence proteins interact with a select group of plant proteins. Some of the plant proteins we defined were already known to be important to the immune system, but others were defined to be involved in development. How immunity and development are balanced remains an important question.

Since then, we have continued to identify bacterial virulence factors to understand their function. Graduate student Beth Mole collaborated with Amy Charkowski and Nicole Perna, then, at the University of Wisconsin, Madison to mine Pectobacterium genomes for type III effectors. Postdoc Tatiana Mucyn identified genes co-regulated with type III effectors in diverse P. syringae and found genes for producing novel toxins. Postdocs Ajay Kumar Goel, Michail Iakovidis, and visiting scientist Chiharu Akimoto-Tomiyama from the National Institute of Agrobiological Resources, Tsukuba, Japan characterized P. syringae type III effector HopAM1 for which no interactor was identified in our yeast studies. We found that HopAM1 damages chloroplasts, increases drought tolerance and triggers part of the immune response but we were unable to identify the target host proteins. HopAM1 has some structural similarity to the TIR protein domain found in one class of NLR proteins. Jeff's postdoc, Marc Nishimura (now at Colorado State University), led a collaboration with Jeff Milbrandt of Washington University showing that these TIR domains catalyze the breakdown of NAD+, forming potential signaling molecules. Recently, Ming Guo and Jim Alfano at the University of Nebraska showed that HopAM1 also breaks down NAD+ into the same products. How these cyclic nucleotide breakdown products affect defense triggering is an active area of investigation. It has been exciting participating in the global effort to define the plant immune system and the ways pathogens overcome it to be successful. Over the past 30 years, the field has unraveled the complexity of the plant immune system and used the knowledge to generate genetically altered plants with improved pathogen resistance ready to be used by farmers.

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How did ASPB impact your career?

Plant Physiology and The Plant Cell have been important sources of information throughout my career. I have enjoyed attending several ASPB meetings over my career. I always enjoyed the scientific presentations, and I appreciated the workshops on education as I began my career as a faculty member. The scholarships for attending meetings and the awards for junior scientists are important drivers to promote plant biology. I am happy to contribute to these activities as a member of the legacy society.

What advice would you offer to a young person contemplating a career in plant science research?

Work in a team: The most successful and rewarding situations in my career have been when I was working as part of a team, with each member tackling the problem from a different angle but all working to understand the same question.

Collaborate with other research groups: Our sex determination project depended on the expertise provided by Boris Vyskot and his colleagues at the Czech Academy of Sciences in Brno. Just as valuable were the fresh perspectives, insights, and encouragement they provided. Furthermore, Jeff and I benefitted tremendously from the generously collaborative environment in the field of plant immunity fostered by leaders such as Brian Staskawicz (Berkeley) and Fred Ausubel (Harvard). In every project I have described above, I have had collaborators from at least one other lab. They enriched the project and made it fun and exciting.

Be gregarious: Take the time to chat informally with colleagues. Great ideas can come from informal chats with people who are not familiar with your work.