Teh-hui Kao

How did your career in plant sciences get started?

Actually, chemistry was my first love, and I received both my B.S. and Ph.D. degrees in Chemistry. It is a long story about how I first fell in love with chemistry and then found a new love in plant biology. My father had a huge influence on my interest in chemistry. He majored in chemical engineering at National Taiwan University, took several chemistry courses with chemistry majors, and became good friends with them. Some of these good friends later became professors in the Chemistry Department of National Taiwan University. While taking science courses in senior high school in Taiwan, I didn't like math or physics, because I thought they were too abstract, and I didn't like biology because I thought it was too descriptive, not to mention that we had to dissect frogs in the lab, which I hated. I had a good chemistry teacher, who was entertaining and often used real-life examples to explain chemical principles. So, much to my father's delight, I was admitted to the Chemistry Department of National Taiwan University, my first choice, after a highly competitive college entrance exam. I chose the biochemistry concentration and did my undergraduate thesis research on purification and characterization of a snake venom protein. For my Ph.D. study in the Chemistry Department at Yale, I chose physical biochemistry as the focus of my coursework and conducted my dissertation research in biophysics with Professor Donald Crothers. So, I was never exposed to plant biology up through graduate school. Interestingly, while at Yale, even though the Chemistry Department is only a short walk from the Osborn Memorial Laboratories, I had no idea there were many prominent plant biologists in that building!

After Yale, I continued to do research in biochemistry, doing a brief postdoctoral stint at the now defunct Roche Institute of Molecular Biology in Nutley, New Jersey. It was the early 1980s, dawn of the recombinant DNA era, so I decided to seek another postdoctoral training opportunity to learn this new and exciting technology. I thought where else would be better than the lab of Professor Ray Wu, who had just edited a volume of Methods in Enzymology dedicated to Recombinant DNA (Vol. 68 published in 1979). When I joined Professor Wu's lab at Cornell, his research focused on method development and DNA sequence analysis. For sequence analysis, I was involved in cloning and sequencing several chloroplast- and mitochondrion-encoded plant genes, mostly for evolutionary analysis rather than a functional study. For method development, I was assigned a project aiming to improve the procedure of cDNA library construction, focusing on the step that involved ligating double-stranded cDNA into a cloning vector. I used terminal transferase to add an optimum length of "tail" to the cDNA and a complementary tail to the vector. Accordingly, I was able to construct a decent rat liver cDNA library.

At that time, Dr. June Nasrallah was setting out to identify genes at the *S*-locus of *Brassica oleracea* that are involved in self-incompatibility. She came to Professor Wu's lab to discuss cloning strategies, and I happened to have an opportunity to talk to her. I had never heard of self-incompatibility before, but after she explained to me the genetics of this reproductive strategy, I became fascinated by the ability of many flowering plant species to use this self/non-self-recognition strategy to allow the pistil to reject self-pollen and prevent inbreeding and promote outcrossing. With Professor Wu's permission and blessing, I devoted part-time to the effort of Dr. June Nasrallah and Dr. Mikhail Nasrallah to clone and sequence cDNA encoding a protein they previously identified as a good candidate for the stigma protein involved in discrimination between self and non-self-pollen. As I became increasingly involved in the collaborative project, I became more and more interested in self-incompatibility. I spent countless hours at Cornell's Mann Library searching for old literature and photocopying tons of papers. I was intrigued by the diverse genetic systems that flowering plants have evolved to circumvent inbreeding. With my background in chemistry and biochemistry, I was most interested in understanding the mechanism for self/non-self-recognition between the pollen and pistil.

As the genetic basis for the self-incompatibility system in Solanaceae had long been established from classical genetic studies, I decided to choose this type of self-incompatibility for my independent research in academia. At that time, Professor Adrienne Clarke's lab in Melbourne, Australia had already been studying self-incompatibility in *Nicotiana*, so, I thought it would be best for me to study another solanaceous species. It was fortunate that Professor Maureen Hanson at Cornell was using petunia in her research program, and she happened to have seeds of self-incompatible *Petunia inflata*, which she generously offered to me. A then graduate student in Professor Dominick Paolillo's lab, Anuradha Singh (now Prof. Anu Singh-Cundy at Western Washington University), was also interested in self-incompatibility, so she helped me grow a number of *P. inflata* plants in her greenhouse so I could collect pistils to look for *S*-allele-associated proteins by SDS-polyacrylamide gel electrophoresis. When my family (my wife and the first of our four children) moved to Penn State in August 1986, we put seven *P. inflata* plants in the trunk of our small car, and it was on those seven plants, all of which survived the trip, that I bet the start of my academic career! I was fortunate that my very first grant proposal was awarded by NSF a few months after I settled down at Penn State. Professor Joseph Mascarenhas was then Director of the Developmental Biology Program, and I am forever grateful to him for his belief in me, allowing me to launch my research program in the study of Solanaceae-type self-incompatibility. *P. inflata* turned out to be an excellent species for studying this, and almost 40 years later we are still investigating new and exciting questions! The better we understand the genes involved and the biochemical mechanisms, the more we respect flowering plants for having evolved such a complex and sophisticated genetic system involving so many genes for the sole purpose of preventing inbreeding.

So, my career in plant science didn't get started because I suddenly fell in love with plants! Perhaps serendipity played the major role. The chance encounter and subsequent opportunity to work with Dr. June Nasrallah and Dr. Mikhail Nasrallah on self-incompatibility in Brassica opened my eyes to this fascinating inbreeding-prevention reproductive strategy. However, without Professor Maureen Hanson's advice on the use of *P. inflata* for my future study and generously providing seeds for me to use, I would not have been able to so smoothly and efficiently establish my foothold in plant science research.

What do you consider your most important contribution to plant science research?

Since I joined the faculty of the Biochemistry and Molecular Biology Department in August 1986, the research in my lab has been focused on Solanaceae type self-incompatibility, also known as S-RNase-based self-incompatibility. When I began my independent research on this biological system, I never expected it would be so complicated and so full of surprises and twists-and-turns. This complexity allowed me to devote my entire scientific career to the study of the same biological system, giving me the opportunity to delve deeper into the mystery of this reproductive strategy flowering plants evolved millions of years ago! Many unexpected results opened new avenues of research and led to significant findings. Almost four decades later, I would say that my most important contribution to plant science research is undoubtedly the advancement of our understanding of S-RNase-based self-incompatibility.

I will describe several of my lab's contributions to understanding this type of self-incompatibility in the *Petunia inflata* model system. We cloned and characterized the polymorphic *S-RNase* gene and used gain-of-function and loss-of-function approaches to show it is necessary and sufficient for pistil function in self-incompatibility, thus establishing that the S-RNase gene alone encodes the pistil determinant. We used site-directed mutagenesis to show the RNase activity of S-RNase is essential for the function of S-RNase in self-incompatibility, suggesting the biochemical mechanism of growth inhibition of self-pollen tubes involves degradation of pollen tube RNA. We identified a polymorphic *S-locus F-box* gene (*SLF1*) from sequence analysis of a chromosomal region containing the S_2-allele of *S-RNase* and used an *in vivo* gain-of-function assay developed in my lab to confirm it is involved in controlling the pollen function in self-incompatibility. We used pollen transcriptome analysis to identify 16 additional pollen-specific *SLF* genes in each of two *S*-haplotypes of *P. inflata*, and used coimmunoprecipitation and mass spectrometry to show that all 17 SLF proteins are assembled into similar SCF complexes (a class of an E3 ubiquitin ligase complex) that also contains a pollen-specific Skp1-like protein (named PiSSK1), PiRBX1 (a RING-finger protein), and a pollen-specific Cullin1 (either PiCUL1-P or PiCUL1-B). My lab and that of Dr. Seiji

Takayama in Japan proposed a collaborative non-self-recognition model to explain the biochemical basis of cross-compatible pollination and self-incompatible pollination. This model predicts that, for a given *S*-haplotype, each SLF interacts with a subset of its non-self S-RNases; all SLF proteins that constitute the pollen determinant collectively interact with the entire suite of their non-self S-RNases to mediate ubiquitination and degradation, allowing cross-compatible pollination; none of the SLF proteins interact with their self S-RNase, allowing the self S-RNase to inhibit pollen tube growth and result in self-incompatibility. We used CRISPR/Cas9-mediated genome editing to confirm that PiSSK1 is essential for pollen to be compatible with pistils carrying non-self *S*-haplotypes (i.e., producing non-self S-RNases), thus confirming that PiSSK1 is the Skp1 component of the SLF-containing SCF complexes required for mediating ubiquitination and degradation of non-self S-RNases. We used CRISPR/Cas9 to show that both PiCUL1-P and PiCUL1-B can serve as the Cullin1 component of the SLF-containing SCF complexes, and that both PiCUL1-P and PiCUL1-B specifically function in self-incompatibility. We used CRISPR/Cas9 to show that SLF proteins are solely responsible for the self-incompatibility function of pollen. We used the *in vivo* gain-of-function assay to establish more than 160 pair-wise interaction relationships between SLF proteins and S-RNases, and the results are completely consistent with the predictions based on the collaborative non-self-recognition model. We showed SLF proteins are themselves subject to ubiquitin-mediated degradation by the 26S proteasome, and identified pollen proteins that may regulate the dynamic life cycle of the SLF-containing SCF complexes.

As the pistil determinant is encoded by a single gene, the finding that multiple *SLF* genes encode the pollen determinant was totally unexpected. Moreover, the finding that the biochemical mechanism is via non-self-recognition, rather than the more conventional self-recognition, like lock-and-key, was also a surprise. However, these findings highlight the similarity between S-RNase-based self-incompatibility and adaptive immunity of vertebrates, where many T-cell receptors (analogous to multiple SLF proteins) are required to collectively recognize a wide variety of foreign antigens (analogous to a large number of non-self S-RNases) in order to mount an immune response to destroy them (analogous to the degradation of all non-self S-RNases to allow compatible pollination). Moreover, none of the T-cell receptors should recognize self-antigens (i.e., those T-cells whose receptors interact with self-antigens are destroyed during the maturation of T-cells, a process called negative selection), lest autoimmune disease result. This is similar to the scenario that, for any given *S*-haplotype, none of the multiple SLF proteins should recognize self S-RNase, lest self-incompatibility break down. Thus, my research on S-RNase-based self-incompatibility has wider implications for the self/non-self-recognition process, a fundamental process in biology.

It is satisfying for me to see that over the years since my lab started to study S-RNase-based self-incompatibility many labs embarked on molecular and biochemical studies of the self-incompatibility found in other flowering plant families. Thanks to their collective effort, we now know that among the types of self-incompatibility studied, the S-RNase type is the most common, as it is also found in five additional families: Plantaginaceae (Snapdragon), Rosaceae (fruit trees), Rubiaceae (Coffee), Rutaceae (Citrus), and Cactaceae (Cactus). It is a mystery why the common ancestor(s) of these flowering plant families "decided" to adopt this biochemical strategy to prevent inbreeding, and how they evolved such a sophisticated mechanism involving so many genes.

When did you join ASPB and how has it impacted your career?

I have been a member of ASPB for almost my entire professional career, as I joined in January 1989, only a few years after I became a member of the Department of Biochemistry and Molecular Biology at Penn State (August 1986). For me, it was obvious I should be a member of this flagship professional society for plant biologists (although at the time I joined the society, it was under the old name, ASPP). This affiliation has impacted my career in a number of ways. The journals that ASPB publishes are the go-to journals for my lab when selecting outlets for publishing what we consider significant results that are likely to have broad interest to plant biologists. The Society launched *Plant Cell* in 1989, and my lab published our first three *Plant Cell* papers all in 1994; we published our latest *Plant Cell* paper in 2023, a span of almost 30 years! Papers published in the Society's journals no doubt give authors broader exposure to the plant biology community. Networking during the Society's annual meetings

and regional meetings is also a great benefit.

The success of any research program depends to a large degree on graduate students and postdocs. I always encourage my graduate students and postdocs to join ASPB, and I support their attending annual meetings and regional meetings to present their research. If their posters are selected for oral presentations, this gives them good experience in public speaking to a scientific audience, and a good opportunity to showcase their research accomplishments and my lab's research program. They often come back with fresh ideas for their research projects and are excited to try new methods they learned during the meeting.

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

As a chemistry major in both undergraduate and graduate studies, I never expected I would establish a long scientific career in plant biology! My bold and perhaps somewhat naïve decision to venture into plant biology during my postdoctoral research at Cornell was merely prompted by my then newly discovered interest in self-incompatibility. I was not daunted by my lack of training in plant biology, as I felt that my chemistry background, research experience in biochemistry, and strong foundation in classical genetics, coupled with my extensive reading of the literature in self-incompatibility, could compensate for my deficiencies in plant biology. This turned out to be a very good decision, as throughout this long journey (almost 40 years), I have maintained a strong passion for my research on this fascinating reproductive strategy, and my lab has continued to uncover new questions to study.

For a young person considering a career in plant biology, I would offer the following advice. Do not choose to study plant biology just because you love plants! It is important that you identify a biological system about which you are passionate, which is rich in biological questions that are technically trackable, and which could enable you to establish a long-term sustainable research career. If the biological system can be best studied in plants, or if the system is unique to plants, then you will enjoy your research in plant biology for a long while!

https://academictree.org/chemistry/tree.php?pid=449368

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

I would advise all early career scientists to seek collaborators who complement their expertise and share their interests and values. As an assistant professor I was warned to avoid collaborating. The message was loud and clear: you will get credit only for "independent" work. I mostly followed this advice and was awarded tenure, but it was at the cost of missed opportunities. Since then, I have worked with scientists from around the world and from various disciplinary backgrounds and career stages.

I would also advise that collaborations work best when the project goals and the roles of the partici- pants are clearly defined at the outset. I am grateful to my many collaborators, including those whom I have mentored and those who have mentored me, for enriching me professionally and personally by generously sharing their ideas and their friendship.

Academic Family Tree
https://academictree.org/plantbio/tree.php?pid=806586