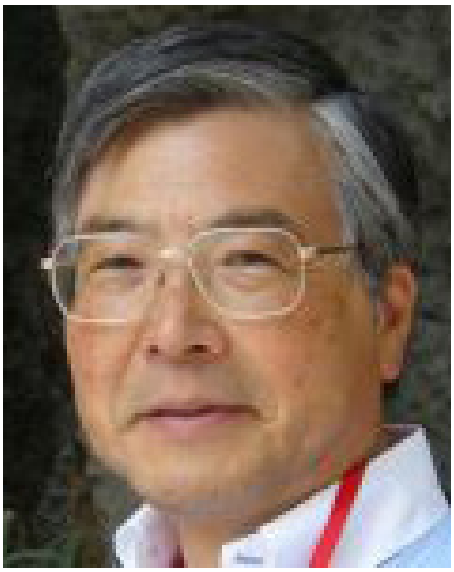
ASPB Pioneer Member

Yuji Kamiya

How did you spend your career?

When I was 14 years old, my chemistry teacher and I had the opportunity to visit RIKEN, Japan's flagship scientific research institute. A young scientist there enthusiastically explained to me the wonders of organic chemistry. Impressed by his lab-coat and passion, I wanted to become a chemist when I went to the university. After graduating from high school in 1966, I entered the University of Tokyo, looking forward to studying chemistry. I did my graduation study at the Bio-organic Chemistry Laboratory in the Department of Agricultural Chemistry. The subject was the chemical synthesis of plant growth inhibitors comparable to abscisic acid (ABA). I synthesized many compounds, but their activities were weaker than natural ABA.

I went on to graduate school and studied biochemistry. There, my research subject was changed to the chemical identification of starfish spawning inhibitors. Dr. Susumu Ikegami taught me the literacy of chemistry. The starfish spawning inhibitor bioassay was carried out at the Ocean Research Institute and the Aburatsubo Marine Experimental Station of the university. I enjoyed the biological experiments along a nice beach and the chemical experiments at the downtown campus. The inhibitor was identified using nuclear magnetic resonance, mass spectrometry, and chemical synthesis as a saponin



having a sulfate group and a sugar moiety in the steroid skeleton. I received my degree in 1975, and I immediately got a research position at RIKEN and became a staff member in the Pesticide Synthesis Laboratory. I synthesized a large number of potential fungicides, but I could not synthesize a compound that was better than fungicides on the market.

Prof. Saburo Tamura, who was the laboratory head, suggested I study a mating/conjugation tube pheromone of the heterobasidiomycete yeast, *Rodospolidium toruloides*, which was isolated at the Institute of Applied Microbiology. This pheromone was chemically quite unstable, and it took me five years to determine its chemical structure. It is a novel peptide pheromone with s-farnesyl cystein at the C-terminus. Later, this prenyl-cystein was discovered to be a very important amino acid in the MAP cascade of signal transduction.

Around 1980, Prof. Nobutaka Takahashi, who was a gibberellin (GA) chemist, became the boss of my laboratory. I told him that I wanted to study the biosynthesis of GAs in plants. With his recommendation, I studied a cell-free system of GA biosynthesis for two years as a Humboldt fellow under direction of Prof. Jan E. Graebe at the University of Göttingen. There, I met Dr. Peter Hedden, who worked as a chemist in Prof. Bernard O. Phinney's (ASPB Pioneer) laboratory. Dr. Wilhelm Rademacher taught me plant physiology. Moving from an organic solvent-smelling laboratory in RIKEN to a plant physiological institute in a beautiful botanical garden increased my research interest in plant science. I studied GA biosynthesis pathways in immature seeds of garden pea. Returning from Germany to RIKEN, I collaborated with a Japanese agrochemical company to investigate the mechanism of a new growth retardant that inhibited GA biosynthesis. The retardant was an environmentally friendly compound and is now widely used in agriculture.

In 1991, I was promoted to PI of RIKEN's International Frontier Research Program. I set the goal for my laboratory to identify the entire GA biosynthetic pathway, clone all the genes encoding GA biosynthetic enzymes, and understand the hormonal regulation of plants. This frontier program was a new and unique research program at RIKEN. I had relatively good

continued on next page

Yuji Kamiya *continued*

financial support and freedom to choose my research subjects. All I needed to do was to open my laboratory to international scientific communities and perform world-wide cutting-edge research. Prof. Dick Kendrick and I created a plant group called Plant Homeostasis, and my team was called Plant Hormone Function. Bernard Phinney, Jake MacMillan, Peter Hedden, Russel Jones, Wilhelm Gruissem, Elizabeth Dennis, Rod King, Lewis Mander, Jan Graebe, Winslow Briggs, Maarten Koornneef and others advised me very often. I started purifying *ent*-kaurene synthase, which catalyzes the first committed step of GA biosynthesis. Radioisotope-labeled intermediates were not commercially available, so I synthesized them myself. Plant *ent*-kaurene is catalyzed by two successive enzymes, namely coparylpyrophosphate synthase (CPS) and *ent*-kaurene synthase (KS). During the purification of these enzymes, Prof. Tai-ping Sun of Duke University reported cloning of the *GA1* gene of *Arabidopsis thaliana* by a genome subtraction method. I thought the function of the *GA1* gene was CPS synthesis based on circumstantial evidence, so I started a collaboration with her and we were able to show that the function of the *GA1* gene really is CPS synthesis. I then focused on the purification of KS and succeeded in cloning the pumpkin KS gene. Both the *CPS* and *KS* genes encode a cyclase with a signal peptide for plastid targeting/transportation. This result provided clear evidence for the location site of GA biosynthesis in plants. Using degenerate primers for plant CPS sequences, I cloned a fungal CPS gene from *Phaeospheria sp*. and *Gibberella fujikuroi*. Unlike the plant CPS, the fungal CPS is a bifunctional enzyme having both CPS and KS activities, and we named it CPS/KS.

Plant GA was believed to be synthesized from mevalonic acid, since it is a diterpene, and a cell-free system prepared from immature pumpkin endosperm effectively converted radioactive mevalonic acid to GAs. One day, Prof. Patricia Leon of Mexico contacted me at the suggestion of Professor Russel Jones, and she asked me to investigate the function of the Arabidopsis *CLA1* gene, which she had isolated. The *cla1* mutant is an albino and needs sugar to grow it in a test tube. To make a long story short, the *CLA1* gene turned out to encode 1-Deoxy-D-xylulose-5-phosphate synthase, the rate limiting enzyme for plastidic isoprenoid biosynthesis. This suggested that plant GA might be synthesized by the methyl erythritol phosphate (MEP) pathway and not by the mevalonate pathway. Using a stable isotope-labelled deoxy-xylulose and mevalonic acid, we could prove that plant GA is synthesized by the MEP pathway and not the mevalonate pathway. By collaborating with a scientist from a different field than mine, I helped make an important discovery in GA biosynthesis.

Physiologically, it is very important to characterize the GA 3b-hydroxylase (GA3ox), which catalyzes hydroxylation of an inactive GA precursor, GA9, to bio-active GA4 at the late stage of GA biosynthesis. Many GA biochemists tried to clone this gene by different approaches. I tried to purify the enzyme from immature bean seeds, and it took several years to do it. Just before I obtained the amino acid sequence of the bean enzyme, the GA3ox gene was cloned and reported by another group using T-DNA tagging of a GA-unrelated gene. I was disappointed, but this experience taught me the power of molecular genetic approaches for cloning target genes.

Phytochromes play important roles during photo-germination of lettuce and Arabidopsis seeds. Red light absorbed by phytochrome induces the conversion of inactive GA precursors to active GAs that promote seed germination, and I worked on this with Shinjiro Yamaguchi (Kyoto University) and Tomonobu Toyomasu (Yamagata University). Degradation of active GAs to inactive metabolites is also important for plant growth regulation. Gibberellin biosynthesis is regulated by many environmental factors including light, low temperature, water content, and nutrients, and it is also strictly controlled by plant development.

In 2000, I was promoted to group director at RIKEN Plant Science Center (PSC), and I could work with more people. In order to

continued on next page

Yuji Kamiya *continued*

understand the multiple hormone networks related to GAs, I collaborated with following people to study the biosynthesis of other plant hormones, especially brassinosteroids (Gerald Bishop and Takao Yokota), abscisic acid (Eiji Nambara), auxins (Tomokazu Koshiba, Hiroyuki Kasahara and Yunde Zhao), strigolactone (Shinjiro Yamaguchi) and cytokinins (Hitoshi Sakakibara).

The PSC group established a plant hormone analysis platform. It is necessary to analyze plant hormone levels accurately and with high levels of sensitivity in order to understand mutual interactions among them. Dr. Sakakibara and my team set up GC-MS (gas chromatography -mass spectrometer), LC (liquid chromatography)-MS/MS and Capillary Electrophoresis -MS using stable isotope-labelled internal standards, and we collaborated with many domestic and foreign plant physiologists. Sometimes more than six LC-MS/MS were in full operation for plant hormone analysis at PSC. RIKEN became known as a center for plant hormone analysis.

Starting from 2000, I was an international advisor for the Annual Review of Plant Biology (previously Plant Physiology and Plant Molecular Biology) for 10 years. It was a new experience for me, and I could learn past, present and future directions of plant physiology research by attending the annual board meeting in California. At that time, information about genome sequences and gene expression in *Arabidopsis thaliana* and rice was available for everyone through the internet, and we entered a new era of the molecular biology of plant hormones.

In 2013, during the reorganization of the PSC, I retired, and my laboratory was replaced by younger researchers. But I had two new jobs. One was the coordinator of a Center for Sustainable Resource Sciences at RIKEN. I gave advice to coordinate young PIs and researchers. I attended the weekly progress reports of some young PI's laboratories and asked friendly questions. The other job was as a helper to the RIKEN administration office for international collaboration since there were few people with scientific degrees in that office. I helped with the Global Summit of Research Institute Directors, which was a satellite meeting of the Science and Technology Society forum held in Kyoto every year. Some of the presidents or heads of research institutes were Nobel Prize winners, and it was fun for me to talk with them about international roles and the problems of research institutes.

Even though I closed my laboratory in 2013, every year from 2014 to last year (2020) I was listed as a "Highly Cited Researcher" by Thomson Reuters. This is due to publications of my collaborators, and I appreciate them very much. I remember that I met Professor Joseph Varner, who discovered the induction of α-amylase in cereal seed aleurone layers by GAs. He said that after he retired from his university, he enjoyed teaching science to kids. I also feel it is very important to teach the next generation. I retired from RIKEN in 2018, and since then I have been teaching science privately to junior high and high school kids through a Non-Profit Organization. Many of my students come from single-mother families, and they have financial difficulties. Sometimes they feel disparities and prejudices at their school. Currently, I am teaching them science on-lines due to the Corvid-19 pandemic. I would like to continue teaching as long as possible.

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

My team and I characterized the biosynthesis of gibberellins, abscisic acid, brassinosteroids, auxins, and other plant hormones using organic and analytical biochemistry. This enabled us to understand the biosynthesis and regulation of plant hormones at the molecular level. We also established a platform that can quantitatively analyze all of the plant hormones with high sensitivity. In the near future, I hope that all the hormones from a single plant cell can be analyzed accurately so we can understand the localization and transport of plant hormones.

What advice would you give to a young person starting a career in plant biology?

When you are young, I suggest you have a wide range of research interests. Of course, it is important

continued on next page

Yuji Kamiya *continued*

to concentrate on your current research, but sometimes advice and collaboration with people unrelated to your research field will help you breakthrough difficult problems. Always try to add something new or an attractive approach to your work and set a goal that no one achieved before. Make scientific friends globally. Never give up on difficult problems. Good friends will surely help you.