

ASPB Pioneer Member

Kazuo Shinozaki

When I was a student at Utsunomiya High School in Japan, I decided to be a scientist. I was very interested in protein as an important biomolecule to understand what life is. I studied biology as an undergraduate at Osaka University (BSc 1972). I was interested in molecular biology, especially in the essential roles of DNA and gene functions. I studied DNA replication in the group of Prof. Reiji Okazaki and Prof. Tsuneko Okazaki in graduate school at Nagoya University, investigating RNA priming of the Okazaki fragment during discontinuous DNA replication. My PhD thesis was on the topic of 'Discontinuous DNA replication of T7 bacteriophage'. I learned the importance of originality and logic in research while working in the Okazaki laboratory.

During my graduate training, I was very interested in the application of recombinant DNA technology for molecular cloning of eukaryote genes. When I was appointed a Research Associate at the National Institute of Genetics (NIG) in Mishima, Japan in 1978, I started to analyze chloroplast genes by DNA cloning in Dr. Masahiro Sugiura's laboratory, where I used molecular cloning to analyze the chloroplast genes involved in photosynthesis. While at NIG, I married Dr. Kazuko Yamaguchi, who studied the molecular biology of plant viruses. Kazuko and I moved to Nagoya University with Prof. Sugiura to study chloroplast genes and the chloroplast genome.



Prof. Sugiura and I determined the complete nucleotide sequence of the tobacco chloroplast genome in 1986, and I was promoted to Associate Professor at the Center for Gene Research at Nagoya University. The chloroplast genome project laid the foundation for my later focus on Arabidopsis functional genomics and the use of reverse genetics approaches in my research project in RIKEN. Prof. Sugiura provided strong leadership for sequencing the chloroplast genome and later discovered novel genes with various functions. We found many homologous genes in the chloroplast and the genome of cyanobacteria, which supported the symbiotic origin of chloroplasts from ancient cyanobacteria. Prof. Sugiura often advised us about the importance of identity and originality for pioneering works in science.

When I worked in Prof. Nam-Hai Chua's Laboratory at Rockefeller University in 1987, Kazuko and

I used transgenic technology to analyze transcriptional regulation of plant nuclear genes. While in New York, we learned about Arabidopsis as a promising model plant for genetics and genomics. I was impressed with the international research setting in the Chua laboratory. Most of the postdoctoral fellows in the Chua lab later became leaders in plant science research.

In 1989, I was appointed Chief Scientist (PI) of the Plant Molecular Biology Laboratory at RIKEN Tsukuba Life Science Center Japan, to start molecular analyses of plant environmental responses using Arabidopsis as a model plant. Kazuko and I decided to start new projects to understand the molecular basis of plant responses to complex abiotic stresses, especially drought, cold, salinity, and heat. We tried to isolate many drought-inducible genes (named RDs and ERDs) with various functions and analyze the regulation of gene expression in abiotic stress responses. We focused our work on transcriptional regulation of abiotic stress responses and their associated signaling networks. We discovered many important genes involved in plant responses to drought, cold, and heat, and analyzed gene expression and signal transduction in abiotic stress responses. We showed for the first time there are ABA-independent regulatory systems in the plant response to drought stress, in addition to those that are ABA-dependent. We

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discovered an important cis-acting element, DRE (Dehydration Responsive Element), in the promoter region that relates the drought stress response and the transcription factor, DREB (DRE Binding protein). Kazuko moved from RIKEN to the Japan International Center for Agricultural Science (JIRCAS) at Tsukuba as Chief Scientist in 1993, and then was promoted to professor at the University of Tokyo in 2004. Since 1989, Kazuko and I have been collaborating to understand complex molecular mechanisms of gene expression and signal transduction in plant responses to abiotic stress.

In 1999, I started an Arabidopsis functional genomics project at RIKEN Genomic Sciences Center (GSC). In this project, we collected full-length cDNAs, transposon-tagged mutants, and cDNA over-expression lines (core project members are Dr. Minami Matsui, Dr. Motoaki Seki and Dr. Takashi Kuromori), and analyzed the transcriptome in abiotic stress responses. The genomic resources and information are available at the RIKEN Bio Resource Center through the continuous effort of Dr. Masatomo Kobayashi. I am pleased with the success of Arabidopsis functional genomics project at RIKEN, which provides tools for the plant science community with collected genomic resources and databases. In 2005, I was appointed Director of the RIKEN Plant Science Center (PSC), the successor of Dr. Tatsuo Sugiyama, and led plant functional

genomics, including metabolomics and hormone functions, as described in the PSC memorial book (http://www.csr.riken.jp/jp/about/publications/documents/psc13_jp.pdf). From 2013 to 2020, I worked as Director of the RIKEN Center for Sustainable Resource Science (CSRS) and led interdisciplinary research projects between biology and chemistry to contribute to sustainable production of biomass, foods, materials, and energy. Now, I support Dr. Kazuki Saito, Director of RIKEN CSRS, as a Senior Advisor (<http://www.csr.riken.jp/en/>). As a project leader, I am pleased with the success of the RIKEN Plant Science Center, which was one of the leading centers in plant science in the 2010s.

I have been on the Thomson Reuters list (later Clarivate Analytics list) of highly cited researchers in the category of Plant and Animal Science since 2007, and in 2011 I was selected 'Hottest Researcher' (most cited) by Thomson Reuters. In 2016, I received two big prizes in Japan: Person to Culture Merit and the Purple Ribbon Medal. In 2020, I received the International Prize in Biology and was elected an international member of National Academy of Science of USA. I would like to take this opportunity to thank all the current and former members of laboratories who collaborated in my research and development. In particular I express my gratitude to Prof. Kazuko Yamaguchi-Shinozaki for fruitful discussions and productive collaboration over a long time to forward research and development.

My major research areas at

RIKEN are molecular biology and functional genomics of plant environmental stress responses and tolerance, and chloroplast functions in stress responses. Major accomplishments from our group include the discovery of essential key regulatory genes and factors such as DREBs, AREBs, MAPKs, and SnRK2s that extend our understanding of plant regulatory systems in environmental responses. We reported an osmosensor, AtHK1, in Arabidopsis. Moreover, we discovered key genes affecting ABA regulation during drought responses, such as ABA biosynthesis (NCED3), ABA degradation (CYP707A3), and ABA transport (ABCG25). We discovered the CRE25 peptide is a long-distance signaling molecule that moves from roots to shoots during drought stress. These discoveries were accomplished through the continuous efforts of my colleagues. I am proud of my productive collaboration with Prof. Kazuko Yamaguchi-Shinozaki that led to the discovery of important cellular regulatory gene networks and our contributions to the advancement of plant stress biology. Crosstalk between plant growth and responses to drought stress at the whole plant level is an important subject if one aims to improve crop plant performance and yield. Translation of basic research to plant breeding and cultivation of stress-tolerant crops is an important challenge to food security during global warming. We can obtain promising results in field trials of drought tolerant transgenic

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crops by international collaboration with foreign crop researchers.

My first priority in research is to understand complex gene networks involved in plant responses and the plant's adaptation to environmental changes – not only at the cellular level but also at the whole plant level. During my career in plant molecular biology, breakthrough technologies took

place every 10 years. These include transgenic plant technology in the 1980s, Arabidopsis genetics in the 1990s, genomics in the 2000s, and functional genomics in the 2010s. In the 2020s, rapid progress in genome editing and high-throughput genome sequencing is becoming a new frontier in life science. Moreover, data science and artificial intelligence is providing powerful tools for the analyses of “big data”, including omics studies and

phenotyping. My advice to young researchers is to challenge one's own ideas and be aware of new technologies that could expand research outputs. Originality and challenge are most important to advance research and development in science. These challenges by the younger generation will open the way to understand complex regulatory networks of plant environmental responses and adaptation as a whole.