ASPB Pioneer Member

Masahiro Sugiura

During my boyhood, we were miserable after the Pacific war (1940-1945), followed by the extreme difficulty of obtaining food. People were exhausted. However, in 1949, Professor Hideki Yukawa at Kyoto University received the Nobel Prize in Physics, the first Nobel Prize winner in Japan. This news encouraged the weakened Japanese people, including me. I admired scientists, and I wanted to be one someday.

Hence, I entered the School of Science at Nagoya University and majored in plant physiology in Professor Yukito Oota's group. I read research articles in journals that came from the US, which were full of recent molecular biology reports. Consequently, I spent every day in a state of frustration. One day, Dr. Syozo Osawa from the next door laboratory came to me and said "you should go to the US and study molecular biology", and he introduced me to Professor Herbert Stern at the University of Illinois. In 1963, I took a long absence from the Graduate School and went to the US. I liked the University of Illinois campus, I felt relaxed and soon my frustration was gone. Dr. Yasuo Hotta was the main person in the laboratory. He kindly helped me adapt to the campus and my new research project. For two years I enjoyed doing experiments on the biochemical analysis of meiosis in lily pollen and campus life with several US students and post-docs.

There was a large molecular biology laboratory on the University of Illinois campus, and Dr. Masaki Hayashi was one of the main researchers there. I often went to see him to ask questions about molecular biology. After one year or so, he was invited to be an Assistant Professor at the newly established San Diego Campus of the University of California. He invited me to join his laboratory as a post-doc. In the summer of 1995, I drove to San Diego by car. It took a week using Route 66.

I was impressed that Professor Hayashi had decided the focus of his life-time research, which was the "Construction of bacteriophage φX174 *in vitro*"; after that he planned to retire. Actually he did! My focus was to establish a coupled transcription-translation system from *E. coli* lysates. Fortunately, I succeeded, and before leaving San Diego I detected i*n vitro* synthesized phage coat protein made from the phage DNA template. A Japanese student in the same department, Mr. Susumu Tonegawa (hereafter, Susumu), worked in the laboratory next door. He later won the Nobel Prize in Physiology or Medicine in 1987. As we were both single, we became good friends.

Dr. Osawa moved to Hiroshima University as Professor, and he kindly invited me to join his group. So, I returned to Japan in October 1966. Professor Mituru Takanami was in the group in Hiroshima, and it was suggested that we work together. We first purified polynucleotide kinase from bacteriopageT4-infected *E. coli* cells. Using this enzyme, we attached P32 at the 5′ end of RNAs. Then, we determined several nucleotides from the 5′ ends of some stable RNAs. This was my first experience with nucleotide sequencing of nucleic acids. Professor Takanami moved to the Institute for Chemical Research at Kyoto University, and I also moved there. I continued to analyze several RNAs using the above methods.

Professor Kinichiro Miura opened a molecular genetics laboratory at the National Institute of Genetics, and he invited me to join him as an associated professor. We started to analyze the 5′ends of several viral double-stranded RNAs, but because these RNAs had some type of unknown structure at the 5′ end, I kept failing for almost 5 years. Later, he and his co-workers found there are cap structures at the 5′ ends of viral RNAs. Professor Miura suggested I develop my own research focus. Soon after, I had a phone call from Susumu at

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the Basel Institute of Immunology in Switzerland, and he asked me to come to his laboratory. I went there during my summer vacation in 1977. During my stay, there was a summer course on gene cloning at Basel University, and Susumu and I attended the evening seminars. I was shocked by the newly developed technology, that is, gene cloning.

I pondered what I should do after my return to Japan. I considered my future research direction could be in many different fields. Most researchers were using bacteria, yeast, and animals (HeLa cells, mouse and so on). But I decided to use plants, because no molecular biologists, to my knowledge, used plants as research materials. Plants are important because almost all organisms on earth depend on compounds produced by them. Plant cells contain three genomes, nuclear, mitochondrial and plastid (chloroplasts), and I finally decided to use chloroplast DNA, because it was known to be a small, simple molecule.

After returning to my Institute, I consulted with plant geneticists. They suggested rice, barley, tobacco, Arabidopsis and so on, and they emphasized that "you should use pure lines". I started using a tobacco pure line species (*Nicotiana tabacum* var. Bright Yellow 4) given by the Institute of Japan Tobacco Inc. Tobacco was a popular model plant at that time, used mostly for cell and tissue culture experiments. I started growing tobacco plants in an old green house and tried to isolate chloroplasts from green leaves and then purify chloroplast DNA. A post-doc, Dr. Jun Kusuda, joined me, and in 1976 we started to clone chloroplast DNA fragments containing ribosomal RNA genes; we published our first paper in 1979. Then, Dr. Kazuo Shinozaki, an Assistant Professor, and several students, including Dr. Fumio Takaiwa, came to our group. Not long after, we cloned other rRNA and tRNA genes. Generally, people determined the nucleotide sequences of their genes (only coding regions), but we sequenced not only the coding regions but also spacers between genes (non-coding regions). Our approach was sometimes criticized as foolish. However, we found the first ribosomal protein gene (*rps19*) in such a region (1983), and hence we decided to annotate even small open open reading frames (ORFs) in sequenced regions. Later, photosynthesis biochemists found that some of the photosystem subunits were encoded in such ORFs.

In 1983, I moved, together with Dr. Shinozaki and his wife, Dr. Kazuko Shinozaki, and two post-docs to Nagoya University as the successor to my advisor, Prof. Oota. I started the laboratory with four graduate students, Dr. Masaru Ohme-Takagi, Dr. Minoru Tanaka, Dr. Nobuaki Hayashida and Dr. Tatsuya Wakasugi. We succeeded in acquiring a large grant, Specially Promoted Research, from JSPS to completely sequence the tobacco chloroplast genome. We created a sequencing facility and an informatics platform to analyze the entire chloroplast genome sequence. After 10 years with over 20 coworkers, we determined the complete nucleotide sequence of the tobacco chloroplast DNA (160 kb, 1986). Our achievement opened the way to study photosynthesis, a major field in plant science, using genomic approaches to identify novel photosystem components. This approach showed the scientific community that to find new genes, complete genome sequencing is more efficient than searching for individual genes of interest one by one. We believe this work has been one of the reasons for supporting whole genome sequencing, and the term "Genome Project" appeared in the 1990s.

We then started to investigate the molecular mechanisms of gene expression in chloroplasts. As the methods were not available, Dr. Tetsuo Hirose, an Assistant Professor, developed *in vitro* (chloroplast extracts) systems that support accurate expression processes: a translation system (1996) and an RNA editing system (2001). An RNA splicing system was developed by post-doc Dr. Keiko Inaba-Hasegawa (2021). These systems provided a breakthrough for molecular and biochemical analyses of precise gene expression reactions. Examples include the identification of precise translation initiation sites, functional identification of *cis*-elements for translation in 5′ untranslated regions (5′UTRs),

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interactions between 5'UTRs and protein-coding regions that control translation activities. Post-docs Dr. Hiroshi Kuroda and Dr. Masayuki Nakamura improved our *in vitro* translation system and allowed us to measure the rate of translation of various mRNAs. Using this system, Dr. Nakamura measured relative translation rates of synonymous codons (2011) and 5' coding regions important for translational initiation (2016). Since chloroplasts originated from an ancestral photosynthetic prokaryote, their gene expression system was thought to be prokaryotic (*E. coli*-type). However, their pre-mRNAs are subjected to RNA editing, splicing and cleavages to produce mature mRNAs for translation, and chloroplast gene expression is mainly regulated at the translational level.

The original sequence (1986) of the tobacco chloroplast genome was updated twice, in 1998 and 2005, and hence the 2005 version is, we believe, the most accurate reference sequence for chloroplast genomes. In addition, we reported for the first time ORF509 (now called *matK*) (1985), and the *matK* sequence has been widely used for plant phylogeny and evolution studies. After tobacco, we sequenced the chloroplast DNA of rice (1989), black pine (1994), *Chlorella* (1997), *Psilotum nudum* (accession no. AP004638), *Nicotiana sylvestris* and *Nicotiana tomentosiformis* (2006), and also the tobacco mitochondrial genome (430 kb, 2005) in collaboration with Dr. Yasuo Sugiyama, an Associate Professor in the Biology Department.

In parallel, Dr. Shinozaki, Dr. Noboru Tomioka and other post-docs isolated (1981) and analyzed several genes of the cyanobacterium, *Anacystis nidulans* 6301 and compared them with the corresponding chloroplast genes, because chloroplasts were thought to have been derived from ancestral cyanobacteria. We reported the first molecular data supporting the endosymbiotic theory of chloroplast origin (1983). Later, we started genome projects of *Synechococcus* sp PCC6301 (formally *Anacystis nidulans* 6301) and *Synechocystis* sp PCC6803. We started both in Nagoya University, and the complete sequencing was done by Dr. Satoshi Tabata of the Kazusa DNA Institute for the latter (1996) and by our group in Nagoya University for the former (2007). We believe we showed that genome projects provide basic data for plant biology research.

Finally, I suggest to young persons that when you become independent, you should decide your lifetime research focus and continue until you reach your final goal.