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Decisions for the future

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Music and science have always been my passion. Ultimately, though, the scale was tipped in favor of science. And I lived out my science dreams in trying to grasp as many facets of it as possible. As a biologist, I didn't stay in one stream and thus, I have embraced not only molecular biology but parasitology as well. This was way back 1980s, when such a research career was viewed highly unusual in a Japanese context. My commitment to malaria and vaccine development follows the same path. I believe one has to integrate knowledge on a wide variety of academic fields—gene expressions, protein structure, epidemiology, immunology—to achieve significant success. Similar to life itself, one has to be prepared well to be able to accept and interpret as widely as possible what one may term as "accidental results" or "accidental elements."

Beginnings

During my high school days, I had two different visions regarding my career path. The first was to enter a music college and study toward becoming a flautist. The second was to study in a science department at a university and ultimately pursue a career as a scientist. I was so serious about my first dream that I even started to learn piano in my final year in high school in order to prepare for the piano skill test which was part of the examinations for entry to music colleges. Eventually, though, my piano teacher advised me not to persist with music studies and to go to a regular university, which was a gentle way of expelling me. As for my second choice, with entrance examinations only a few months away, I was still undecided as to whether to pursue physics or mathematics studies. At that time I also recalled the biology class that I had taken in my first year of high school. It was about some kind of enzyme reactions, and a lecture was given on the feedback control of such reactions. The concept of feedback control originates in electronics. Being an amateur radio enthusiast, as well as a music lover, I had a strong feeling that the subject of feedback control melded physics with biology. I was attracted to this field of science, which used physicochemical approaches to gain an understanding of biology, and then I came across the entrance guide/introductory package for the Department of Biological Sciences in the Faculty of Science at Osaka University. In this department, there were no botany or zoology courses, but we could study biophysical chemistry, biochemistry, molecular genetics, etc. I sensed that I had found my future direction. Finally, the day came when I stood at the door to a career as a molecular biologist.

I moved on to the Department of Biological Sciences, Graduate School of Science, Osaka University, where, from the late 1970s when recombinant DNA technology was in its nascent stage, I became engaged in a molecular mechanism study on homologous DNA recombination using the *Escherichia coli* RecA protein, under Professor Hideyuki Ogawa (currently a professor emeritus). Starting with structural elucidation and expression analysis of the *E. coli* RecA gene, we worked to understand the "mechanism of SOS function," a mechanism of response to environmental mutagens in prokaryotes. Today, DNA sequences can be analyzed through outsourcing. However, back then when determining the sequence

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of the RecA gene, I spent a whole year just to determine the sequence of 1,000 bp, using a very complex method called the Maxam–Gilbert method. At that time, in order to handle DNA, we had to purify nearly all related enzymes ourselves, such as restriction enzymes, kinases and ligases, and this required considerable effort. Still, DNA manipulation was an advanced technology and just having the freedom to handle DNA meant high status. By around 1980, we had started conducting analyses on mutation-induced RecA genes and domain analyses using DNA manipulation. In order to delve deeply into the molecular mechanism of homologous DNA recombination which supports life, it was necessary to elucidate the binding of the RecA protein and DNA, the interaction between RecA proteins, and conformation of the RecA protein. To me, all this was endlessly interesting and at the same time I realized the difficulties inherent in these study topics. That was a quarter of a century ago. Today, protein structure and its function remain as central topics in molecular biology.

After 1980, studies to reveal the phenomena of higher forms of life, such as immunology, became popular. At Dr Ogawa's laboratory, we received recruitment information for postdoctoral researchers from the renowned Nobel Prize-winning scientist Dr Susumu Tonegawa. I had great difficulty in choosing my future research theme. Soon after, we received postdoctoral recruitment information from then Professor Joseph Inselburg at Dartmouth College in the United States. The research theme was on malaria using molecular biology approach. Competent persons who could introduce the technologies, including cDNA library construction, were being sought. I did not want to become a cog in the wheel of a big laboratory, but rather an integral part of a small laboratory. I knew nothing about *Plasmodium*, so I visited the main library of Osaka University and learned about malaria from encyclopedias. In fact, I was planning to take advantage of my time as a postdoctoral researcher to think about a research theme that could become my lifework. I had no intention of it being malaria research.

About Dr Horii

Dr Toshihiro Horii studied Biology and Physiology at Osaka University (Japan). After he completed his PhD in 1981, he stayed in the Department of Molecular Genetics at Osaka University as research associate. From 1984 to 1986 Dr Horii gained experience in the Department of Microbiology at Dartmouth Medical School (NH, USA). A few years after his return to Osaka University, he became Head at the Department of Molecular Protozoology, and since 2005 has also been Director of the Research Center for Infectious Disease Control and International Research Center for Infectious Diseases.

Dr Horii's research focuses on the development of malaria vaccines and drugs. In addition, he and his team are using molecular and cell biological approaches to analyze the mechanisms by which plasmodium adapts to a new host. Other interests include population genetics, meta-genomics and infectious diseases.

Dr Horii has published more than 150 original research papers in various peer-reviewed journals, as well as many review articles and commentaries. He is an active member of many professional societies, including the Japanese Society for Vaccinology, and has received numerous awards and honors, such as the 51st Koizumi Prize awarded by the Japanese Society of Parasitology in 2004.

Serine Repeat Antigen

From 1984 through 1986, I was engaged in malaria research at Dartmouth College. In the first three months, I isolated the SERA (Serine Repeat Antigen) gene of *Plasmodium falciparum*. By the late 1980s, molecular biology had become so popular that it could no longer be called leading-edge science; the field of biomedicine was almost completely dominated by molecular biology. In contrast, research on infectious diseases was on a downward path, and infectious disease-related courses were beginning to disappear from universities across Japan. Back then, even at the Research Institute for Microbial Diseases, Osaka University (RIMD), there was a great deal of discussion about whether or not to maintain the Department of Protozoology following the retirement of a former professor. Ultimately, it was decided to preserve that department and that I would be a candidate to take over as head of department upon my return to Japan. So, in 1991, I accepted the post of chief associate professor. Contrary to my earlier plans, research on malaria was about to become my lifework.

Currently, I work as a parasitologist or as a vaccine researcher, but I aim to be a biologist who reads widely in biochemistry, structural study, genetics, immunology, studies on infectious diseases, and epidemiology. We are trying to develop a vaccine for malaria, an infectious disease, but gene expressions and physical properties of purified proteins are of key importance. Studies in genetic biochemistry and on protein structures, in which I was involved in my undergraduate days, became very useful in this regard. Knowledge of epidemiology

and immunology is also essential. It is not novel for a parasitologist to adopt the methodology of molecular biology and in the United States and Europe, molecular parasitology began to flourish in the 1980s. It has been said that many parasitologists in Japan, back then, failed to follow the trend of molecular biology. I would disagree. Molecular biologists failed to advance into the field of parasitology. I believe that the Japanese tendency to head in the same direction as everyone else or, conversely, to turn away from a certain direction altogether, has prevented the merging of various fields of science. My research career could be viewed as unusual in a Japanese context. However, outside of Japan, there is nothing unusual about such a career path. Without doubt, this difference comes from Japanese culture and social climate. The spirit of "Once you start something, stick to it" can be said to be one of the principles underlying fundamental Japanese culture. Of course, it is important to stick to a study until you are satisfied. However, if someone uses this as an excuse to remain uninterested in other academic fields, he or she inevitably falls behind the progress of modern science. Being interested in a wide variety of academic fields is necessary in order for a scientist to achieve significant success.

I have been working on development of SERA malaria vaccine since 1991. Back then, I was often asked why I did not study well-known vaccine candidate antigens that were being studied by many researchers, such as MSP-1. I opted to keep studying SERA because the more I read related papers, the more I realized that there were various disqualifying data for other vaccine candidate antigens. Meanwhile, as I proceeded with SERA research, I found almost no disqualifying data regarding SERA as a vaccine candidate. Hence, I was not distracted by other vaccine candidate antigens, and have continued on my chosen research path to this day. However, expression of recombinant protein using cDNA of the SERA gene did not work. Here, my knowledge in molecular biology was a great help. Codon usage differs significantly between *P. falciparum* and *E. coli*, so I assumed that, if I were to create an artificial synthetic gene whose codon usage matched that of *E. coli*, a recombinant protein could be prepared. In those days, we had to purchase reagents and synthesize them one by one; and massive research expenditure was required. It was a big decision.

When it became possible to prepare recombinant SERA proteins from *E. coli* using artificial synthetic genes, we were able to conduct various animal experiments, and we obtained many results. For a time, I believed that the effect observed in the laboratory was everything. However, compared to the cunning strategies for evading the host immune system, such as gene polymorphisms and antigenic variations, that *P. falciparum* has developed in the course of its evolution, the wisdom of a scientist seemed insignificant. We later found that the most important factor was whether or not anti-SERA antibodies contribute to the protective immunity acquired through natural infection in people living in endemic areas. In 1997, I received an email message from Dr Thomas Egwang in Uganda, who had read our paper. It contained an offer to engage in a joint research project to investigate if antibodies against the SERA protein that we had purified existed in people who live in highly endemic malarial areas of Africa, and if these antibodies are involved in anti-malarial immunity. We were able to purify an ample quantity of recombinant proteins, so we immediately sent some to Dr Egwang. He had been conducting similar epidemiological studies on various vaccine candidate antigens in collaboration with researchers from the United States and other countries, and he added SERA in their evaluation. After a few months, we received an email message informing us of the results: "There are almost no individuals with malaria symptoms who are SERA antibody-positive. However, there are some healthy individuals who demonstrate high antibody titer." I remember this as the moment when I became bolder and took the courage to push SERA as a vaccine.

BK-SE36 Malaria Vaccine

I got an opportunity to introduce our study on SERA malaria vaccine to Dr Michiaki Takahashi, then a professor emeritus at RIMD and director of the Research Foundation for Microbial Diseases at Osaka University (BIKEN). BIKEN was established in 1934 and has developed vaccines based on the pioneering basic studies conducted in RIMD. It is a vaccine manufacturing organization engaged in the production and supply of numerous vaccines. Dr Takahashi was the researcher who first developed the varicella vaccine. He showed great interest in our study and offered encouragement. This meeting opened the way for the SERA vaccine to proceed to clinical trials. Since then, under the code name BK-SE36, we have gone on to GMP manufacturing, pre-clinical studies, and domestic clinical trials for this vaccine.

With BIKEN sponsorship, we conducted a Phase Ib clinical trial on BK-SE36 malaria vaccine in Lira, Northern Uganda, from 2010 through 2012. The objective of this Phase Ib clinical study was to investigate the safety and immunogenicity of this vaccine. In Stage 1, safety was verified in adults aged 21 years and older with a history of malaria infection. Subsequently, a similar evaluation was conducted in younger population of ages 6 through 20 years, in Stage 2. Sixty-six subjects were vaccinated in this stage and after investigating safety and immunogenicity, we wanted to observe whether or not these vaccinated subjects would be protected against malaria infection. We consulted with BIKEN and was awarded a research grant. After gaining approval from the Ugandan government, we conducted a research study. The result: Compared to the control group, the vaccinated group demonstrated a protective effect of 72%. Based on data analyses of antibody titer responses to vaccination and protective effects, a higher protective effect could be expected if higher antibody titer response to vaccination was achieved through addition of a better adjuvant. I discovered that Dr Ken Ishii, who had been a fellow smoker in the smoking area and with whom I used to discuss the issue of children's day-care centers at the university, was actually an expert on immunoadjuvants. I asked him to participate in the study as a member of my laboratory staff, and launched a study on increasing the immunogenicity of BK-SE36 malaria

vaccine. We initiated animal experiments adding CpG adjuvant to BK-SE36 malaria vaccine, and we have had positive outcomes. Currently, a Phase Ia clinical study is being conducted.

Decisions for the Future

We have to make many choices in our lives, not only as scientists but also in general matters, and these choices shape our lives. It is important to be ready to respond flexibly to chance meetings and accidental results. Louis Pasteur, a scientific genius whose life and work I have the greatest respect, said, "Le hasard n'est utilisé par que l'esprit préparé." This has been commonly translated into Japanese as "Fortune visits only those who are prepared," and into English as "Chance favors the prepared mind," but the original meaning is a little different. "Le hasard" does not mean fortune or chance, but simply coincidence. The inevitable essence hidden in a coincidence can only be used by those who are mentally prepared to interpret it. Whether good or bad, scientists often find unexpected experimental results. Whether or not they can understand the meaning of the coincidence and make use of it in the next experiment depends on how many possibilities they have pondered. I am still unsure if I am fully prepared. Still, within my abilities I know I have opened wide the door of opportunity and accepted as many possibilities as I could.