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From Ca^{2+} in muscle contraction to IP_3 receptor/Ca^{2+} signaling
In memory of Setsuro Ebashi

Professor Setsuro Ebashi passed away on the morning of July 17th, 2006 at the age of 83. Despite a stroke that had left him with left hemiplegia and aphasia, he had participated in the symposium celebrating the 40th anniversary of the discovery of troponin that was held from October 25th to 28th 2005. He made a speech at the party and enjoyed meeting many colleagues, and he was looking forward to publication of the proceedings. Unfortunately, however, he passed away before they were published.

Setsuro Ebashi was a professor of the Department of Pharmacology of the Faculty of Medicine of the University of Tokyo. He contributed to science through his great achievement of elucidating the mechanism of muscle contraction by purifying troponin C and also identifying the relaxation factor, the sarcoendoplasmic reticulum. He also inspired me to continuously and actively devote myself to the pursuit of science. Professor Ebashi was originally a pharmacologist, but his research included biochemistry, physiology, and neurochemistry. His work had a great impact not only in the field of pharmacology, but of biochemistry, physiology, and other branches of science. In the field of biochemistry, he was a pioneer in research on molecular interactions, on the structure-function relationships of proteins, and on function in cell science. Although the subject of his research was muscle, his research was not limited to the study of muscle but extended to neurochemistry, as clearly demonstrated by his later becoming a professor of the Division of Neurochemistry of the National Institute of Physiological Science. I heard Professor Ebashi speak on many occasions, but I was especially impressed by his Plenary Lecture at the meeting of the Japanese Biochemical Society just before his retirement from the University of Tokyo and his talk upon his retirement, since they were so different from the ones that other professors gave. His achievements in science were already enormous, but he expressed his ambition and wishes to continue his research. He continued his research with his wife, Dr. Fumiko Ebashi, at the National Institute for Physiological Science in Okazaki City, which is located near Nagoya, and even after retiring from the National Institute for Physiological Science he conducted research by himself in a laboratory supported by pharmaceutical company.

Not only did Professor Ebashi do great work in revealing the mechanism of muscle contraction, especially on the role of Ca^{2+} in muscle contraction, but he always inspired many young people and gave them great encouragement and ambition to pursue science. The work initiated by Professor Setsuro Ebashi not only had a great impact on our research on the role of Ca^{2+} in muscle contraction, but in other tissues as well. He also gave us insights into various approaches to clinical science into human muscle abnormalities such as muscular dystrophy. Many researchers in Japan have identified the genes responsible, and their work was promoted by the great influence of Professor Setsuro Ebashi.

When I started my work, Professor Ebashi was already a renowned researcher in bioscience and a leading scientist worldwide. It is amazing that he became a professor of the Department of Pharmacology at 36 years of age, succeeding Professor Kumagai. A new book entitled 'Molecular Biology' was published in 1963 by the Asakura Publishing Company in Japan. That was the time of a new ascendance of biophysics in Japan which included structure biology and molecular biology in addition to biochemistry and physiology. The editors of the book included Professors Masao Kotani, Fujio Egami, Itaru Watanabe, Yonosuke Ikeda, and Fumio Oosawa, and Professor Setsuro Ebashi wrote about excitation-contraction coupling and explained the importance of the endoplasmic reticulum. When I was still a medical student at Keio University School of Medicine I repeatedly read his published articles, and they sparked my interest not only in biochemistry but in biophysics as well. The close scientific connection on the subject of calcium between Professor Ebashi and myself started when I was a Ph.D. (doctoral) candidate doing research in the Department of Physiology of Keio University School of Medicine.

The department consisted of two laboratories, one headed by Professor Tsuneo Tomita and the other headed

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by Professor Yasuzo Tsukada. Professor Tomita contributed to demonstrating the existence of three absorption peaks in the carp retina, confirming the three pigment hypothesis. I was greatly influenced by Professor Tomita and his colleagues by learning the importance of electrophysiology. The other laboratory, headed by Professor Tsukada, was engaged in research in the field of neurochemistry.

Professor Tsukuda succeeded Professor Emeritus Takashi Hayashi, who discovered that GABA is an inhibitory substance and that glutamate is an excitatory substance, now called a neurotransmitter. The laboratories had regular joint meetings and a journal club meeting every week. Since I had an affinity to molecules through biochemistry, I was interested in neurochemistry.

Although I was studying and working in a different university, I was influenced by Professor Ebashi through listening to his talks and reading many of his articles. In medical school, there was a program to instruct students in the conduct of physiology experiments. When I entered the Department of Physiology as a Ph.D. candidate, I became involved as one of the members of the education group. There were several subjects of the laboratory course work, but most of them involved classical physiology experiments. The work by Setsuro Ebashi's group designed to elucidate the mechanism of muscle contraction and Ca^{2+} regulation interested me, since it strongly impressed me that muscle contraction mediated by Ca^{2+} is an ideal model for teaching medical students about mechano–chemical coupling at the molecular level. Since I really wanted to carry out a muscle contraction experiment, I persuaded Professor Tsukada to include the subject of muscle contraction in the laboratory course work for the medical students. I carefully read many elegant works published in papers written by Professor Ebashi and colleagues and pondered how I could clearly demonstrate the dynamic nature of the mechano–chemical coupling mechanism of muscle contraction at the molecular level to students. I first obtained the actomyosin fraction from rabbit skeletal muscle and performed 'actomyosin thread' experiments, 'superprecipitation' by adding Mg^{2+}, Ca^{2+}, ATP in the presence of EGTA or EDTA. I also remember introducing the technique of the glycerol-treated skinned fiber experiment developed by Professor Reiji Natori. In this preparation of what skinned fiber, the medical students and I also analyzed the contraction detected by the kymographion when we added Mg^{2+} and Ca^{2+} in the presence of EGTA or EDTA. It was a very rewarding experience for me to see the young students excited by the data they obtained in the experiments, and it was important for them to see for themselves that muscle contraction occurs in vitro. At that time I was involved in isolating neurons and glial cells from the rat cerebral cortex, and I never expected to be working in the field of Ca^{2+} signaling. The knowledge I gained during the early stage of my career in research on Ca^{2+} helped me a great deal in carrying out my later work on Ca^{2+} signaling, and I am very grateful to Professor Ebashi who introduced me to Ca^{2+}.

I would now like to explain why I became involved in research on Ca^{2+} signaling. I had the opportunity to work in the laboratory of Professor Jean-Pierre Changeux at the Pasteur Institute in Paris. He was very famous for having discovered allosteric transition. At that time, I was conducting research on cerebellar mutant mice that displayed abnormal behavior. I was working on the P400 protein, which is abundant in cerebellar Purkinje cells and deficient in Purkinje-cell-deficient mutant mice, that is, *nervous*, *pcd* mutant mice. Mutants with poor dendritic arborization lack P400 protein. Several years after returning to Japan, I was elected to be a Professor of Osaka University, Institute for Protein Research. I asked professor Changeux if I could start studying P400, and he encouraged me to proceed. After a long struggle, I found that P400 protein is related to the molecule, a pharmacologically hypothesized IP_3 receptor, that regulates IP3 to release Ca^{2+} from non-mitochondrial stores. It took a long time to discover that P400 is the IP_3 receptor and that the storage site is the endoplasmic reticulum. The knowledge I learned from Professor Ebashi taught me a great deal about how to approach the most important target among the various phenomena in science, based on the example of the role of Ca^{2+} in muscle contraction. Therefore, as soon as I and my colleagues discovered that P400 is the IP_3 receptor that is important in releasing Ca^{2+}, we immediately planned and performed experiments on the role of Ca^{2+} in cell function.

When I was asked to organize a laboratory as a Professor (adjunct position) of the National Institute for Basic Biology, the NIBB, in Okazaki, I was very pleased to accept, because at that time Professor Ebashi was President of the National Institute for Physiological Science, which is located next to the NIBB. Whenever, I visited the NIBB, I spent a long time discussing the results obtained by my younger colleagues. It was often midnight when I returned to the residence owned by the Okazaki Institutes, and I almost always met Professor Ebashi and his wife on way home after finishing their experiments. I was always impressed and inspired by their positive and ambitious attitude with regard to research.

When Professor Setsuro Ebashi turned 70, symposium was held at the Physiological Institute of Okazaki to celebrate the occasion. Since at that time we had discovered and cloned the entire cDNA of the IP_3 receptor and established that the receptor is a Ca^{2+} channel and IP_3 binding protein, Professor Ebashi kindly invited me to deliver a lecture at the symposium. I have enclosed here a photograph of all of the members who participated in the celebration of his great achievement. A memorial symposium on Ca^{2+} signaling has been organized for next year, and will be supported by the Takeda- Foun dation.

When Professor Makoto Endo, who succeeded Professor Ebashi as Professor of the Department of Pharmacology retired, a symposium was held in honor of Professor

Endo. I was selected to be one of the three speakers, another of whom was Professor Ebashi. It was a great honor and memory for me to have delivered a lecture at the same symposium with Professor Ebashi.

I had the opportunity to organize the Congress on Ca^{2+}-Binding Proteins and Ca^{2+} Function in Health & Disease at Kisarazu in Chiba Prefecture in Japan. I asked Professor Ebashi to participate and deliver the Plenary Lecture, which was scheduled to last an hour, and he kindly accepted. More than 350 scientists from all over the world participated, and the program was nicely organized. He frequently asked highly appropriate and pointed questions. He delivered a great talk on the history of his research on Ca^2 including his great work on discovery of troponin, and a relaxation factor in the sarcoendoplasmic reticulum, and recent work, including work on the 3D structure of the Ca^{2+} pump by his pupils, including Professor Chikashi Toyoshima. Many young researchers from abroad were very stimulated and excited.

Whenever Professor Ebashi thought of new ideas or questions, he immediately called the colleagues in his laboratory, even if it was in the middle of the night. I, too, often received calls from him, and he asked me many questions. I phoned him when I was asked to be a Professor of Department of Physiology of the School of Medicine Keio University and of other Universities, and he clearly pointed out the advantages and disadvantages for me and for the universities that offered me the position. His suggestions were very helpful to me in deciding whether to remain at the University of Tokyo or move to Keio University or other universities. I ultimately stayed on at the University of Tokyo, and that decision determined the future direction of my research.

It has been 20 years since I discovered the $P400/IP_3$ receptor and my work extended into the area of Ca^{2+} signaling. However, I was always able to directly contact Prof. Setsuro Ebashi and ask his opinions. I was also selected as a member of the selection committee of prize winners of some of the foundations where Professor Ebashi was a chairman. That opportunity allowed me to become very familiar with how he evaluated Investigators' scientific achievements of investigators.

I would like to express my appreciation for encouraging me and for everything Professor Ebashi has given me.

Celebrating Setsuro Ebashi's 70th birthday

National Institute for Physiological Sciences, Okazaki, Japan

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At the 39th Yamada conference

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