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REGULATORY MECHANISMS OF STRIATED MUSCLE CONTRACTION

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PREFACE

The discovery of troponin by Professor Setsuro Ebashi opened a new era for research into the regulation of striated muscle contraction. This volume is the proceedings of the symposium held at Okazaki, Japan, in 2005 celebrating the 40th anniversary of that discovery.

Professor Ebashi started his work on muscle contraction when he was a young researcher, immediately after World War II, having been inspired by the book *Chemistry of Muscular Contraction* by Albert Szent-Györgyi. He was fascinated by the dynamic features of the contractile processes performed by the two contractile proteins, myosin and actin, in the presence of ATP. However, he wondered about the mechanism by which muscle relaxes after contraction. He proceeded with biochemical studies of muscle relaxation and found in 1952 that a factor present in the supernatant of the suspension of minced frog skeletal muscle caused relaxation of glycerinated muscle fibers. Based on this finding and succeeding work, he came to the conclusion that the relaxation of contracted muscle was caused by the uptake of calcium ions from the cytosol into the relaxing factor (sarcoplasmic reticulum). His work greatly contributed to elucidating the entire processes of excitation–contraction coupling, particularly the role of calcium ions in triggering the contractile response of myofibrils.

Then he found that superprecipitation of actomyosin, i.e., an in vitro contraction model, became sensitive to calcium ion concentration in the presence of a protein factor other than myosin and actin. This factor showed some similarity to tropomyosin, which had been reported by Bailey, and thus was called native tropomyosin.

When I started working in Professor Ebashi's laboratory in 1963 as a graduate student, he was working very hard with his wife to characterize the biochemical properties of native tropomyosin. Tropomyosin, reported by Bailey, was actually isolated by isoelectric precipitation from native tropomyosin, but it did not confer any calcium sensitivity to actomyosin. In 1965, Professor Ebashi succeeded in isolating a new protein named "troponin" from native tropomyosin, which, in association with tropomyosin, made actomyosin sensitive to calcium ions. With a series of studies in the 1960s, the molecular basis for the regulation of muscle contraction was established. Since that time, troponin has been the central object in research on the regulation of striated muscle contraction. Properties of troponin, which consists of three different components, have been extensively investigated as revealing insights into a representative calcium-receptive and calcium-regulatory protein.

Professor Ebashi and I sincerely hoped that this volume would become a milestone for future developments in the study of the regulation of muscle contraction and related biomedical sciences. I would like to express my profound gratitude to all contributors for their heartfelt cooperation.

Finally, I regret to say that Professor Ebashi passed away in July. Although he could not see this volume through to publication, every detail of the book should be in accord with his long-lasting interest in muscle science. He was a great pioneer in the molecular biology of the regulation of muscle contraction.

Iwao Ohtsuki
August 2006

October 19, 2005

Dear Ebashi-san,

On this anniversary of the discovery of troponin, I would like to add a few words to all the praise of this great achievement – in a letter, since to my great regret I cannot be at the symposium in person.

What in my eyes made this work so outstanding and so successful was that it was all based on one concept: calcium triggers muscle contraction. Also, your interpretation of this concept was unusually broad for its day, extending calcium signaling beyond myofibrillar shortening to other processes essential to muscle contraction as a whole, such as the rapid release of fuel at the beginning of contraction. To show what I mean I would like to trace the elegant progression of this work in a few words.

When I first met you in 1958 or 1959, you had just started to look for evidence that calcium was the activator of myofibrillar contraction. If it was, you reasoned, relaxation should be caused by calcium removal from myofibrils and actomyosin. This implied that the relaxing factor should act by removing calcium from myofibrils. Thus, in Lipmann's laboratory you were searching for calcium binding by what was then called the particulate relaxing factor. More than that, you were searching for ATPase-dependent calcium binding because you had shown earlier that the relaxing action of the relaxing factor disappeared when its intrinsic ATPase activity was destroyed. You found what you searched for.

During this same period I heard you give a seminar discussing the other aspect of this argument: myofibrils should relax whenever calcium was removed; for instance they should relax in the presence of chelating agents. In 1961 you published a paper that demonstrated this. The concept that calcium removal from myofibrils was responsible for relaxation was new: do you remember how it was roundly rejected by nearly everybody in the field at the 1962 Dedham Conference?

The basis for the rejection was that a mixture of highly purified actin and myosin – in contrast to myofibrils or actomyosin extracted as the complex between the two proteins – always contracted on ATP addition, even after the complete removal of calcium by EDTA. However, you persisted, because you knew you were right. The experiments with purified actomyosin drove you to search for a calcium-sensitive structural protein that might have been removed during the preparation of actin. In 1963 you announced in *Nature* that "native tropomyosin" was the protein responsible for the calcium regulation of actomyosin contraction. In 1965 you, Fumiko [Ebashi] and Ayako Kodama succeeded in isolating the small protein that made native tropomyosin different from pure tropomyosin and was responsible for calcium sensitivity. You named this protein troponin, and some years later Iwao Ohtsuki in your laboratory showed that it was bound to the I-bands of the myofibrils.

The evidence that calcium signaling extended beyond myofibrillar shortening came in 1967, when, in collaboration with Eijiro Ozawa and Keizo Hosoi, you showed that the

low calcium concentrations that initiated actomyosin contraction simultaneously also activated phosphorylase kinase, thereby triggering the explosive breakdown of glycogen, which supplies the fuel for contraction. In my recollection this was the first time that a messenger was shown to integrate a program rather than control a single reaction.

Ten years after the beginning of your series of discoveries, in 1968, you and Makoto Endo put together the whole calcium story in muscle in a beautiful review discussing in depth the role of calcium in every aspect of muscle contraction. Do you remember the feeling of doom during the student riots, when you wondered whether the review might be your last paper? I vividly remember your fear that the students might completely destroy your laboratories and thus put an end to your scientific career.

This did not happen, and instead your laboratory became one of the centers of muscle research, attracting frequent visitors. I remember fondly the enthusiastic group of young collaborators and students surrounding you, the freshness of the discussions, and the extremely generous hospitality we all received. It was a wonderful time.

I wish I could have told you this in person.

Annemarie Weber

The most important development in muscle science since the 1950s has been the discovery of the troponin–tropomyosin system and the light it has thrown on the mechanisms involved in the regulation of contraction in striated muscle. Indeed, I believe when the mechanism of regulation is completely elucidated it will lead to a further understanding of the interaction of actin and myosin itself. In view of Setsuro Ebashi's outstanding discovery and the further contributions of many of his colleagues, it is entirely appropriate that the celebration of the fortieth anniversary of this achievement should be made in Japan.

It grieves me very much that because of the current state of my health it would not be wise, and would certainly be very uncomfortable, for me to make the long journey required to attend this important meeting. Because of my long interest in this field I find this hard to bear. It is the one meeting I would wish to attend and renew acquaintance with many friends and colleagues of long standing. To them I convey my good wishes.

As one of the few muscle biochemists to visit Japan soon after the war, I was fortunate to meet in 1953 the individuals who were beginning to lay the foundations of the outstanding Japanese contributions to muscle science. Associated with Hiroshi Kumagai was a promising young scientist, Setsuro Ebashi, who was sedimenting out a relaxing factor fraction from muscle homogenates. Koscak Maruyama was a somewhat precocious graduate student and Fumio Oosawa and Yuji Tonomura were laying the foundations of their careers. My visit was somewhat unconventional, for I was enjoying a six-week tour of Japan as the Manager of the Cambridge University Rugby Team.

Setsuro Ebashi once commented to me that as rugby players we were treated like princes whereas life was very hard for him and his colleagues. It was clear from the visits to laboratories and muscle scientists made when I could sneak away from my duties that the funding of Japanese universities and research was still suffering from the war. I was, however, impressed with what had been achieved with very limited resources. Perhaps the most impressive event witnessed during my visit was Reiji Natori's demonstration of the skinned fibre preparation, then unknown in the West. In his laboratory he had a single muscle fibre mounted under a drop of oil. After sharpening a matchstick he amazed me by running it carefully along the fibre, detaching the membrane and leaving the myofibrils exposed.

Iwao Ohtsuki has made important contributions to the structure of the troponin complex and we are fortunate that he and his colleagues have organized this meeting at such an appropriate time. He deserves our sincere thanks for all his efforts and the efficient way he has carried them out. May I hope that when the meeting ends we shall have a mechanism for the molecular function of troponin with which we all agree.

S.V. Perry
October 19, 2005