CC/NUMBER 45
NOVEMBER 5, 1990

This Week's Citation Classic ®

Ebashi S & Ebashi F. A new protein component participating in the superprecipitation of myosin B. *J. Biochem. Tokyo* 55:604-13, 1964; and **Ebashi S, Kodama A & Ebashi F.** Troponin. I. Preparation and physiological function. *J. Biochem. Tokyo* 64:465-77, 1968.
[Dept. Pharmacology, Fac. Medicine, Univ. Tokyo, Japan]

My original intention was to prepare "native" actin that together with myosin would constitute Ca^{2+}-sensitive actomyosin. This was accomplished by the procedure described in the first paper, but the native actin was found to be accompanied by another protein system, which resembled tropomyosin in many respects but did not have the same biological activity as classical tropomyosin. Ca^{2+} sensitivity of natural actomyosin or myosin B is ascribed to the presence of native tropomyosin in the actomyosin system. This "native" tropomyosin can be extracted from muscle mince directly without acetone treatment. The second paper describes the method of separating troponin from native tropomyosin and some of its properties as the Ca^{2+} receptor protein. The conclusion that troponin is the sole Ca^{2+} receptor of the contractile system, with four receptor sites, is based on the experiments with hybrid actomyosin systems that are composed of myosin, actin, tropomyosin, and troponin derived from either skeletal or cardiac muscle. [The *SCI*® indicates that these papers have been cited in more than 345 and 390 publications, respectively.]

Reaching Tropomyosin at Room Temperature

Setsuro Ebashi
National Institute for Physiological Sciences
Myodaiji, Okazaki 444
Japan

June 8, 1990

The atmosphere of the Conference on Biochemistry of Muscle Contraction, 1962, held in Dedham, Massachusetts, the largest muscle symposium up to that time, was extremely unfavorable for the Ca concept. This led me to the conclusion that I must answer the question of why pure actomyosin was not sensitive to Ca^{2+}. Annemarie Weber, the sole tovarich in the campaign for Ca, had already suggested that Ca^{2+} sensitivity might reside on the actin side.[1] I thought that actin in the natural state should be sensitive to Ca^{2+}, but the conventional procedure to use acetone for preparation of actin would destroy this sensitivity; the key experiment in my view must be to prepare native actin. On the way home from the conference, I stopped in New York and told Weber my resolution. She enthusiastically agreed with my idea and encouraged me.

Returning to Japan, I tried various procedures to extract actin from muscle mince without acetone treatment, but none was successful. I then extracted actin from the mince that was still drenched with acetone, skipping the step of drying the acetone-treated mince. Surprisingly, this simple modification enabled me to obtain Ca^{2+}-sensitive actin, and I at once concluded that my hope had been fully realized.

I had to discontinue this work temporarily, but after three months I took it up again together with my wife. To our great disappointment, we were unable to duplicate the previous result. After a month of seemingly hopeless struggle, I suddenly realized that the earlier experiment had been made in the midst of Japan's severe summer, and I had not been particularly careful to keep the preparation in a cold state; our second attempt had been made in the comfortable fall climate with strictly controlled temperature at nearly 0° C. We then carried out the extraction at room temperature and were able to reproduce the results obtained four months prior.[2] For a muscle scientist, this finding straightforwardly indicated the involvement of tropomyosin, and since that time, there has been no difficulty in obtaining the native tropomyosin described in the first paper and then in isolating troponin from this.[3]

The next natural step was to measure the Ca binding of troponin, and the data for it were almost completely already in 1966. In spite of this, I hesitated to conclude that troponin was the Ca^{2+} receptor protein in the contractile machinery. Looking back, I cannot be certain why I was so timid, but there may have been two reasons at that time: One was the overwhelming belief of muscle biochemists in myosin, i.e., myosin had a leading position in any contractile process, and I could not get rid of such a belief that myosin would then also be involved in the process of Ca^{2+} regulation; the second, and perhaps more important one, was a lack of confidence in myself—it was unbelievable to me that such a humble scientist as I could have made such a discovery.

My reluctance was overcome by the hybrid experiments that utilized the different Sr^{2+} sensitivities between skeletal and cardiac troponins, making absolutely unquestionable the conclusion that troponin is fully responsible for the Ca^{2+} regulation of muscle contraction.

1. **Weber A & Winicur S.** The role of calcium in the superprecipitation of actomyosin. *J. Biol. Chem.* 236:3198-202, 1961. (Cited 240 times.)
2. **Ebashi S.** Third component participating in the superprecipitation of 'natural' actomyosin. *Nature* 200:1010, 1963. (Cited 175 times.)
3. **Ebashi S & Kodama K.** A new protein factor promoting aggregation of tropomyosin. *J. Biochem. Tokyo* 58:107-8, 1965. (Cited 185 times.)

1A-17