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A period of convergence in the studies on muscle contraction and relaxation: The Ebashi's contribution

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Abstract

The object of this paper is to trace the growth of a fundamental problem that for a decade hindered the development of several lines of muscle research: the molecular mechanism that allows and controls contraction and relaxation of muscle fiber. Emphasis is placed on the difficulties to be overcome; thus the paper records not only the achievements and successes, but also the unavoidable failure and disappointments. The account highlights the essential contribution of Setsuro Ebashi to find the solution of the problem.
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There are periods in the history of science in which different lines of research seem to converge towards a common problem that hinders their development because the problem cannot be solved by means of concepts and instrumentation of each individual lines. The exit is unpredictable since, for a scientific solution to be formulated successfully, a concerted interaction of many events must occur. If the solution is reached, then the convergence point marks a catalyzing moment for the unfolding of unexpected problems and the opening of new fields of researches.

An emblematic phenomenon of convergence occurred at the end of 1950s in the studies of muscle contraction and relaxation. In the very same years, the developments of muscle physiology, biochemistry, and ultrastructure were hindered by the same intricacy: the molecular mechanism that allows and controls contraction and relaxation of muscle fiber. It has been my privilege to live and work through that period. My participation in research and observation of the relevant progress that has taken place in muscle physiology is my justification for accepting the invitation of the Editors to write an account of that period for a better understanding of Ebashi's contribution.

Accounts of the period we have under review, point out three events: the observation of inward spread of action potential toward the center of the fiber; the re-discovery of an almost forgotten component of muscle; the finding of a "relaxing factor" in muscle homogenates. The importance and the significance of these events can best be appreciated in relation to the difficulties they generated.

A physiological problem: the inward spread of activation in muscle fiber

In the beginning of the 1950s, attention of electro-physiologists has been focused on the difficulty of explaining the rapidity with which an action potential brings the contractile material into action. This difficulty was pointed out on both theoretical and experimental grounds by A.V. Hill [1,2]: the problem was how the excitation travels inwards the myofibrils. A great advance in understanding of this problem has been made by Andrew F. Huxley and Robert E. Taylor who found that a reduction of membrane poten-

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tial is not effective at all points on the surface of the muscle fiber, but causes contraction only if it affects certain sensitive spots [3,4]. In muscles where the spots are placed along the attachment of the Z lines to the sarcolemma, when the membrane potential at one of these spots are reduced, the corresponding I band shortens and the shortening may spread a considerable distance inward, toward the center of the fiber, without affecting the adjacent sarcomeres. Clearly, the inward spread of activation in local stimulation experiments could not be explained by simple diffusion; yet the phenomenon appeared not understandable in the absence of any recognition of the existence of an anatomical basis within the fiber. Mention of sarcoplasmic reticulum seemed to have almost disappeared from text books, reviews, and other papers dealing with structure, function and biochemistry of muscle [5]: the sarcoplasmic reticulum, described in detail by Veratti in 1902 [6], had became "a virtually forgotten component of muscle" [7].

A morphological problem: the re-discovery of an almost forgotten component of muscle

In the early 1950s, the William L. Bragg's Memorandum to MRC 1947 [8] had made the students of biology aware of the need of filling the gap between the level of organization which the chemist can analyze by his methods and those which the microscopist can see. Behind this aim laid the hope of demonstrating a causal chain of evidence from the molecule through to morphological structure and function. In this perspective, the development of electron microscopy was giving a new interpretation of the cytoplasm; the several faults, that had impeded a clear interpretation of the observations, stimulated the search for better materials and methods.

The history of the re-discovery and gradual unfolding of knowledge about the sarcoplasmic reticulum has been recently reviewed by Clara Franzini-Armstrong [9] and by Paolo Mazzarello and coworkers [10]. The pioneering electron microscope investigations of Stanley Bennett and Keith Porter [11], Keith Porter and George Palade [12], and of Ebba Andersson-Cedergren [13] revealed the complex organization of the sarcoplasmic reticulum and its characteristic three-component structures, the triads, arranged with a geometrical relation with the cross-striations of myofibrils. The connection of the tubular element of the triads—the T-system—with the surface membrane, suggested by the observations of Porter and Palade [12] and of Andersson-Cedergren [13] remained, however, largely hypothetical. In view of this uncertainty and of the lack of any information regarding the enzyme properties of this structure, the suggestion advanced by Stanley Bennett [5] that the intermediate element and the morphological system of which is a part, might be involved in the inward spread of action potential, remained a stimulating working hypothesis. No attempts have been made to suggest a functional significance of the proper sarcoplasmic reticulum, that is, after all, a prominent structure closely applied to myofibrils [14].

A biochemical problem: the puzzle of the "relaxing factor"

In the early 1950s the basic conditions for muscle contraction—interaction of actomyosin with ATP and high ATPase activity—have been established with certainty. It was also established that, under certain conditions, high concentrations of ATP and low ATPase activity may lead to dissociation of actin from myosin, i.e. may induce "relaxation". The question then arose: what keeps the unstimulated muscle in the relaxed conditions?

In 1951 Marsh [15,16], working on muscle homogenate, observed that the removal of the supernatant from the fiber fragments and resuspension of the washed fibers in 0.16 M KCl, led to a very striking increase in ATPase activity of the fibers and to an immediate contraction on addition of ATP. He postulated the presence in the supernatant of a factor which he termed "relaxing factor", protein in nature and capable of inhibiting actomyosin ATPase. Marsh also observed that on addition of 2 mM CaCl to fresh fiber suspension only shrinkage was possible; he related this effect to direct Ca^{++} stimulation of actomyosin ATPase [16]. Attempts of characterizing the physical and biochemical nature of the relaxing factor led to rather conflicting results. Initially, the suggestion has been widely accepted that the relaxing activity was in fact the expression of the presence in the supernatant of an ATP-generating system [17–19]. The proposal has proved incorrect by Portzhel [20] and by Bendall [21] who convincingly demonstrated, by the use of differential centrifugation, that the relaxing activity was associated with a vesicular fraction, operationally defined as microsomal particles or "granules". These findings agreed with a previous observation by Kumagai, Ebashi, and Takeda [22] that a particulate fraction, identified as a component of the relaxing system, was similar to the Mg^{++} dependent "granular" ATPase described by Kielley and Meyerhof [23].

However, several authors pointed out that, under certain conditions, the relaxing activity seemed to be associated to a diffusible material rather than to a vesicular fraction [24–26]. Moreover, it was found that the relaxing activity of vesicles could be stimulated by addition of a soluble cofactor obtained by spinning down the granules [27,28]. In view of these conflicting observations, the eminent British muscle biochemist Dorothy Needham, concluded her detailed review on the subject: "How exactly the Marsh factor comes in and out of play is one of the most obscure problem at the present time" [29].

Overcoming the impasse

Two series questions were open by the researches on the relaxing factor, i.e. the identification of the subcellular structure(s) from which the "granules" could originate and the mechanism of its relaxing effect.

In most of original works on the relaxing factor, little if any attention was paid to the problem of its intracellular origin. The idea that the relaxing fraction from muscle homogenate might originate from fragmentation of sarcoplasmic reticulum was tacitly assumed on the basis of morphological analogies since, on the electron microscope inspection, the fraction appeared to consist of vesicular and tubular structures bearing some resemblance in dimensions with those of sarcoplasmic reticulum [30–36]. Incidentally, it is quite astonishing that reference was often made to ''endoplasmic reticulum'' rather than to ''sarcoplasmic reticulum'' on analogy with microsomes from other cell types. It is however important to notice that the techniques then available did not allow to draw unambiguous conclusions merely from morphological observations. The resolution attainable did not reveal structural details to distinguish vesicular fragments deriving from the different membranous structures of muscle fiber. Moreover, studies by differential centrifugation have shown that a significant amount of relaxing activity, even greater than that of microsomes, could be found also in the mitochondrial fraction [37]. Thus there was an area of uncertainty as to whether there was a unique type of particle in muscle which was responsible for relaxation [38,39]. As a matter of fact, reliable identification of the intracellular membranous structures from which, upon fragmentation, the relaxing fraction might derive, deserved the vesicles be characterized to a far greater extent in terms of biochemical properties than in terms of morphological description. My own contribution was to demonstrate, by means of integrated biochemical and morphological criteria, that the membrane fraction having all the enzyme properties of the relaxing factor, did not derived from fragmentation of mitochondria, but solely from fragmentation of sarcoplasmic reticulum [40–43]. These results were confirmed by the use of zonal centrifuge, a technique that made it possible to achieve a fractionation of subcellular particles with very high resolution [39].

The realization that the sarcoplasmic reticulum contained an enzyme system involved in muscle fiber relaxation, and the observation that the T-system was the structure responsible for the inwards spread of action potential [44,45], led to the conclusion that this complex system of tubules and vesicles was certainty implicated in the excitation–contraction coupling, although the sequence of molecular events leading from a wave of depolarization to myofibrillar contraction, remained obscure.

Questions about the mechanism of the relaxing activity have been mostly addressed by analyzing the effect of relaxing factor on Mg^{++}-activated myofibrillar ATPase. The proposal that the essential feature of the action of Marsh factor was its inhibition of Mg-activated ATPase activity of myofibrils was first analyzed experimentally by Portzhel and her collaborators [20,46,47]. They also found that the inhibitory effect of the factor was completely lost in the presence of 0.1 mM calcium ions, thus confirming the previous observation by Bendall that very low concentrations of calcium ions were enough to prevent the relaxing effect of Marsh factor [48]. These findings were also in agreement with the original observations by Bozler that low concentrations of calcium ions down to 0.1 mM, abolished the relaxation of muscle fibers, an effect He interpreted as due to stimulation of myofibrillar ATPase activity [49,50].

The longstanding attention of muscle physiologists to the effect of calcium ions on muscle contraction was vividly renewed by the studies of Annmarie Weber who convincingly demonstrated that a very small fraction of Ca^{++} is required for myofibrillar ATPase and contraction [51]. Almost simultaneously, similar results were obtained by other authors [34,37,52,53]. It was also established that the behavior of actomyosin systems in the presence of ATP depended on the concentration of ionized calcium: if calcium is omitted, superprecipitation does not occur and the actomyosin gel dissolves under the influence of ATP [52,54]. The straightforward conclusion drawn by Ebashi from these remarkable results was that the relaxing factor could act simply by binding the fraction of free Ca^{++} required for high myofibrillar ATPase and contraction [55]. The possibility that the activity of Marsh factor could be account for by its capability of binding metal ions necessary for ATPase activation, was already considered by V. Perry [56] and was indirectly suggested by the effect of the metal–chelator EDTA on muscle fiber relaxation [57]. But the proposal has proved correct experimentally by Ebashi. The long series of studies in Ebashi's laboratory afforded support for the view that the ATP-linked calcium-accumulating activity of the relaxing fraction, represents the mechanism of the relaxing action of the factor on actomyosin system, showing, as they did, the close relation between calcium uptake and relaxing effect [34,35,53,55]. The question then arisen: which is the molecular mechanism by which the vesicles bind free calcium ions, and why only in the presence of ATP?

Heterogenesis of a solution

Before Skou began his research in 1957, the existence of concentration gradients for Na^+ and K^+ across plasma membrane was well established, but difficult to understand in view of the documented permeability of the membrane to electrolytes. To explain this paradox, in 1941 Dean proposed that the plasma membrane contain an active pump [58]; but the proposal did not receive experimental attention. It was also known that the transport of both cations across the membrane is active and that the energy required comes from hydrolysis of ATP [59,60]. Starting from these observations, in 1957 Skou demonstrated that the membrane-bounded ATPase activated by Na^+ and K^+, is in fact the enzyme responsible for the transport of these cations across the membrane [61]. To understand the impact of Skou's discovery, it is worthwhile remembering that, besides the specific interest for cell physiology, the ATPase discovered by Skou was the first example of a cation-activated transport ATPase, and its identification gave substance for the first time to the concept that the transport

across the cellular membrane could be mediated by specific membrane-bound protein structures. Since the work of Skou, the attitude to Dean's concept had become much more sympathetic and the concept of energy-requiring pump was receiving frequent approving mention in literature. The demonstration by Hasselbach and Makinose [36,62] and by Ebashi and Lipmann [35] of the existence of an energy-requiring system for the transport of calcium ions into the tubules and vesicles of sarcoplasmic reticulum and that this ''calcium pump'' was responsible for the control of calcium concentration within the fiber, is somehow related to the development of these concepts.

The convergence point

The right interpretation of the mechanism of calcium-binding by sarcoplasmic reticulum and the almost simultaneous demonstration of a material continuity of the membrane of T-system with the surface membrane of the fiber, marked the convergence point of the different approaches to the study of muscle contraction and relaxation. The fundamental problem that hindered the development of muscle researches, i.e. the molecular mechanism that allows and controls contraction and relaxation of muscle fiber, was overcome. The sarcoplasmic reticulum was recognized as the anatomical basis that allows the spread of action potential inwards the myofibrils and its control of Ca^{++} concentration within the fiber as the mechanism of its regulatory function of muscle contraction and relaxation.

The success of this synthesis was beyond most expectation. In the fifty years which have elapsed since Ebashi substantially contributed to understanding the molecular mechanism of muscle contraction and relaxation, a very complex story has unfolded and the story has taken many unexpected turns to which again Ebashi gave invaluable contributions [63–65].

In conclusion, I would like to point out that this account was not intended to be a complete and detailed history of a certain field of research; rather it focus on the dynamic role played by the particular form of scientific development here described as ''convergence of lines of work''. The report emphasizes on the difficulties to be overcome; thus it records not only the achievements and successes, but also the unavoidable failure and disappointments. Yet the history of the development of our knowledge on muscle contraction and relaxation may helps to understand that efforts of establishing priorities in time and value, should be regarded as a superficial exercise: every scientist is an instrument of history, everything he has learned and everything he attempts to do are product of history, and everything he does or publishes becomes history.

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