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Skinned Fibers and Natori's Staircase

Shigeru Takemori
Department of Physiology, The Jikei University School of Medicine

In the early spring of 1949, out of the chaos of the postwar years, Reiji Natori (Fig. 1) successfully removed the cell membrane from an isolated fiber of frog skeletal muscle. His accomplishment was a great challenge to the conventional beliefs of the day. Most physiologists and physicists believed that the cell represented the smallest unit of life. Accordingly, the cell was considered the separable limit of a living organism. Decomposition beyond this point was believed to destroy biological function in an irreversible manner. Natori's challenge preceded the great breakthroughs in a new era of biological sciences. Hodgkin and Huxley[1] published their work on the mechanism of nerve excitation in 1952, Watson and Crick[2] proposed the double-helix DNA model in 1953, and Hugh and Andrew Huxley proposed the sliding theory of skeletal muscle in 1954[3,4].

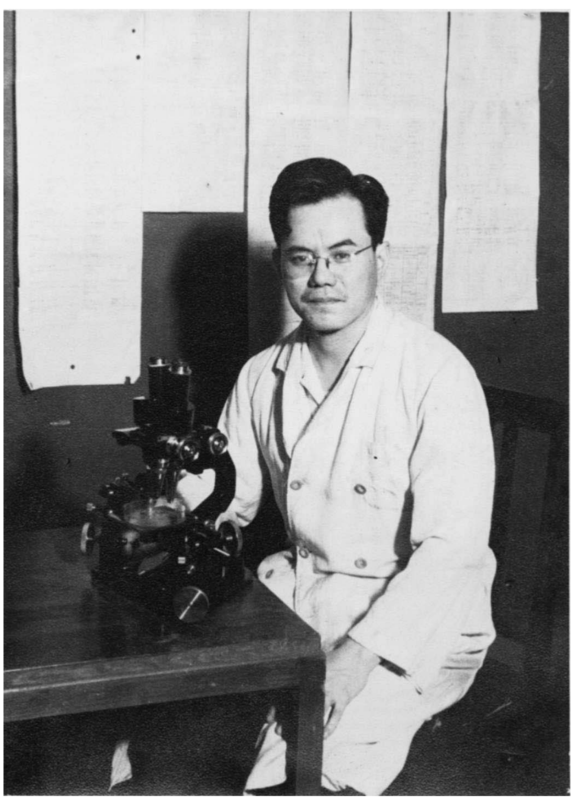

Fig. 1. Reiji Natori the year he successfully invented the skinned fiber preparation (1949). That same year, he became a professor of the Department of Physiology and worked hard to establish the Japanese Society of Physical Fitness and Sports Medicine.

Earlier days: Growth with a great interest in physics

Reiji Natori was born in Tokyo in 1912. During his childhood, knowledge in the field of quantum mechanics advanced rapidly. For example, when Natori attended *Doitsugaku-Kyoukai* Junior High School, Heisenberg[5] proposed his uncertainty principle. The principle suggested an inevitable limit for the accuracy with which the position and movement of any object could be determined. This proposal shocked those who believed in the determinism of classical mechanics as well as the entire scientific community. Analogical consideration led several scientists, such as Niels Bohr[6], to argue that there is an inevitable limit for analytical approaches to the understanding of life. In fact, when a living organism decomposes to its chemical elements, such as carbon and hydrogen, all the information about their functional roles in life will be lost. Combined with the fact that cells separated from the 2- and 4-cell embryos of sea urchins develop normally[7], the argument set the probable limit for the separation of life at the level of the cell. This argument strongly attracted young Natori, who very much loved physics. He took private lessons from a theoretical physicist when he was a medial student at The Jikei University School of Medicine.

Upon graduation from the university, Natori began his studies of physiology with Professor Seizaburo Uramoto. Natori was soon attracted to the study of the mechanical properties of skeletal muscle. With progress in his studies of living muscle fibers, Natori began to feel that experimental evidence obtained through the cell membrane was insufficient to draw rigorous conclusions. Natori began to suspect that decomposition of a muscle fiber, which is a cell, was ultimately necessary for substantial progress in the scientific understanding of muscle. However, this suspicion conflicted with the belief he still held that decomposition of a cell would destroy the components essential for biological function.

Skinned fiber : Successful demembranation in oil

After the war, few experimental resources remained in the laboratory. Sophisticated measurements of the cell interior through the cell membrane no longer seemed possible. So, to study the myoplasm directly while putting the decomposition limit aside, Natori started to work with minced muscle ejected from the needle of a syringe. The minced muscle thread actually contracted and relaxed under certain conditions. However, he suspected that the contraction of the thread was unrelated to physiological muscle contraction, which is a biological function. Therefore, he decided to return to the cell and took a more careful decomposition step. He tried to carefully remove the cell membrane of a living muscle fiber under a stereomicroscope using a knife made from a broken razor blade.

Since it was only in late 1950's that Ebashi[8] determined that Ca^{2+} induces contraction of skeletal myoplasm, Natori never thought to remove contaminant Ca^{2+} from the solution. For this reason, Natori's every attempt to remove the cell membrane in an artificial salt solution was unsuccessful. Once the cell membrane was damaged, the myoplasm began to contract without relaxing until all the fine structures had been destroyed. Then Natori thought, "O.K., let the myoplasm keep its own fluid after the removal of the cell membrane. Since oil does not mix with the aqueous intracellular solution, removal of the cell membrane in oil may work." His idea turned out to be correct. He was able to remove the cell membrane of a muscle fiber while leaving the myoplasm in a relaxed state. This was the first skinned fiber. The fiber responded with a transient reversible contraction to the application of an aliquot of artificial salt solution. This result indicated that the fiber retained the physiological nature of myoplasm as far as contractility was concerned. Natori was relieved by this success.

He reported his invention of the skinned fiber preparation at the 26th annual meeting of the Physiological Society of Japan held in Kyoto[9]. Natori summarized his observations with skinned fibers in a series of reports published mainly in the Jikeikai Medical Journal[9-13].

Evidence for the internal excitatory membrane system

Because excitation was already known to be a transient potential change across the cell membrane, Natori initially expected that electrical stimulus would elicit no response in skinned fibers. So he was surprised to see that the skinned fiber responded to electrical pulses with propagating waves of contraction[12-14]. The propagation started from the anodic side of the electrodes, in contrast to the ordinary excitation of living muscle that starts from the cathodic side. When a cut was made in the middle of the skinned fiber along its length to split a part of the fiber into two branches, the contraction wave elicited on one of the branch propagated to the other branch at the root of the branches. From these observations, Natori concluded that there is an excitable membrane system in the myoplasm and that the electrical potential of the membrane is inside out. The membrane system is now considered to comprise T-tubules and sarcoplasmic reticulum.

Elastic element of the myoplasm : Connectin/titin filaments

Skinned fibers revealed another astonishing feature of myoplasm that could not be observed through the cell membrane : extreme extensibility with almost complete reversibility[11]. Skinned fibers can be extended to more than three times the resting length. The extension limit of living muscle is usually much shorter because the short extension limit of the cell membrane and the surrounding connective tissue causes the inhomogeneous extension of the myoplasm to rupture the fiber.

When the main constituent of myoplasm was shown to be the interdigitation of the lattices of thick myosin filaments and that of thin actin filaments, Natori predicted that there should be another elastic component linking the lattices longitudinally. Later Maruyama and Kimura[15,16] found a gigantic elastic protein named connectin in skeletal muscle. This elastic protein is now called connectin/titin. It is so large that it spans more than 1 μm in length and has been shown to constitute a third filament system in myoplasm, as Natori had predicted.

Because skinned fibers lack a cell membrane, which limits diffusion from the incubating solution, one can easily control the solution milieu for the myoplasm. This particular feature enabled various experiments to investigate the nature of the contractile apparatus and internal membrane systems in the fields of physiology, pharmacology, and biophysics[17–21].

Natori's staircase: an implicit research project

Despite the remarkable success of the removal of cell membrane, Natori dared not rush down the decomposition process. He carefully examined the nature of skinned fibers, seriously considering the possible differences caused by the removal of the cell membrane. Natori[22] usually explained his views using a staircase (Fig. 2). He placed the cell at the top of the staircase and the elementary proteins at the bottom. Biochemists and biophysicists are trying to climb the stairs by integrating the properties of the elements, such as myosin and actin, to construct an understanding of cell function. Natori placed himself, as a physiologist, at the top of the staircase, trying to descend one step at a time, minimizing the irreversibility of each step. The reversible part will be ascribed to the nature of the decomposed parts, while the irreversible part will not. When researchers ascending the staircase meet the descending physiologists in the middle, they can construct an integrated understanding of the cell. This type of implicit research project is a natural outgrowth of the historical development of human science.

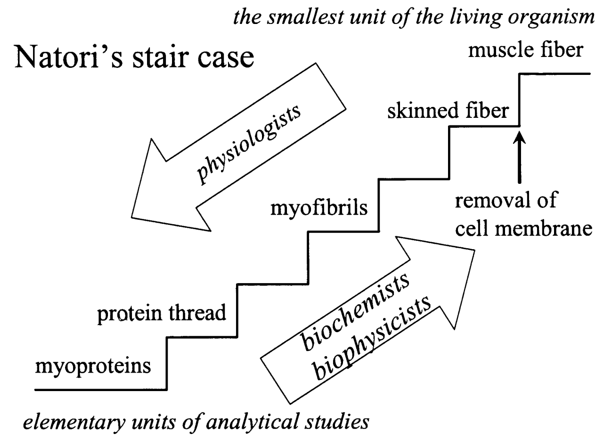

Fig. 2. Natori's staircase representing the implicit research project in biological sciences.

Natori's love for living creatures

Natori felt somewhat anxious to see that muscle physiology at top of the staircase was not advancing as rapidly as biochemistry and biophysics at the bottom. Natori still believed in the limit for analytical research in the biological sciences. Studies of decomposed elements are certainly a necessary part of life science. They powerfully ascribe some parts of life to the elements of the organism. However, there is no reason to believe that all biological functions can be ascribed to the elements. Some biological functions may emerge only at the levels of living organisms; that is, cells, tissues, organs, individuals, and societies. Therefore, even if there is a possibility that a particular aspect of life is ascribed to the nature of a decomposed part of the organism, studies at the levels of living organisms must help the sound development of biological sciences (Fig. 3).

Natori often amused us by saying, "Have a drink as soon as you get an expected result in your experiment, and have a drink as soon as you get an unexpected result in your experiment." He sometimes added his reasoning thusly: "Never repeat the experiment right away. Or you will probably unconsciously distort the evidence to fit your hypothesis, leaving your reservation for the spoiled properties of the experimental preparation."

Fig. 3. Natori continued his experiments on skinned fibers even after he became an emeritus professor. In his experiments he did everything himself, even the washing of used glassware. He stopped his research on the afternoon of June 15, 1994, when he failed to mount a skinned fiber onto the experimental apparatus. He never asked anyone to assist him. An integral part of Natori's experiments was to feel the working of life with his own hands.

Out of his belief in the necessity of studies at the levels of living organisms, including both individuals and societies, Natori dedicated his best efforts to the development of sports medicine and physical fitness. In fact, in the very year he invented the skinned fiber preparation, Natori actively promoted the establishment of the Japanese Society of Physical Fitness and Sports Medicine.

He also dedicated his efforts to the development of The Jikei University School of Medicine and Tokyo Jikeikai. He very much loved his societies, including his country, academic circles, university, and family. All his life was beautifully designed by himself in harmony with his sincere respect for the working of life at every level, from cells to his country.

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Appendix : A brief chronology concerning Reiji Natori (1912–2006)

1912	0 yr	Born in Tokyo.
1923	11	*The Great Kanto Earthquake*
1928	15	*Heisenberg publishes an essay on the uncertainty principle*
1929	17	Graduates from *Doitsugaku-Kyoukai* Junior High School
1933	21	*Bohr publishes "Licht und Leben"*
1936	24	Graduates from The Jikei University School of Medicine
		Began researches on physiology with Professor Seizaburo Uramoto
1945	33	*Pacific War ends*
1949	37	Invented the skinned fiber preparation
		Became a professor of physiology at The Jikei University School of Medicine
		Establishes the Japanese Society of Physical Fitness and Sports Medicine.
1952	40	*Hodgkin and Huxley publish mathematical formula for action potential*
		Hosts meeting on muscle physiology (*Kinnseiri-no Tsudoi*)
1954	42	Publishes a series of papers on skinned fibers in Jikeikai Medical Journal.
1959	47	*Ebashi identifies* Ca^{2+} *as the physiological activator of muscle contraction*
1964	52	Organizes International Congress of Sports Sciences in Tokyo
1973	61	Receives Medal with Purple Ribbon.
1975	63	*Maruyama purifies connectin from skeletal muscle*
		Becomes president and the chairman of the board of trustees of The Jikei University School of Medicine.
1977	65	Becomes emeritus professor
		Receives Asahi Prize
1981	69	Receives Japan Academy Prize
		Named Person of Cultural Merit by the Japanese government
1986	74	Receives Order of Cultural Merit from the Japanese government
1988	76	Becomes a member of the Japan Academy
1992	80	Receives the Grand Cordon of the Order of the Sacred Treasure
2006	94	Dies of cancer