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Birth of the Sliding Filament Concept in Muscle Contraction

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Why were the two classical papers by A.F. Huxley and R. Niedergerke and by H.E. Huxley and J. Hanson on the sliding filament concept in muscle contraction published in the same issue (May 22, 1954) of *Nature*? This historical survey reveals the background of the two groups' monumental work.

Key words: Andrew Huxley, Hugh Huxley, Jean Hanson, muscle contraction, Rolf Niedergerke, sliding filament concept.

In the 1954 May 22 issue of *Nature*, two classical papers appeared: "Structural changes in muscle during contraction" by A.F. Huxley and R. Niedergerke (*1*) and "Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation" by H.E. Huxley and J. Hanson (*2*). It is generally accepted that these two short papers presented the first evidence for the sliding filament concept in muscle contraction (*3, 4*).

By that time it was a prevailing opinion under the influence of English biophysicist William Astbury (1898-1961) and Swiss chemist Kurt H. Meyer (1883-1953) that muscle contracted as a consequence of conformational changes of the filamentous proteins (*5*). For example, wide-angle X-ray diffraction work by Astbury and Dickinson had suggested that the changes of the structures of muscle contractile proteins from the β form (β sheet) to the α form (α helix) occurred during muscle contraction (*6*), although this change was later denied by Astbury himself in 1947 (*7*). Morales presented a primitive type of the sliding concept in 1948 (*8*).

The sliding filament concept was revolutionary at the time, and is now regarded as a major universal mechanism of biomotility, not only in myosin/actin-based motility but also in the kinesin, dynein/microtubule-based motility of cells. A molecular motor (myosin, kinesin, and dynein) slides along a rail (actin filament or microtubule) in a definite direction dictated by the polarity of the rail, utilizing the energy derived from the splitting of ATP (Fig. 1a). When the motors are appropriately immobilized, then the rail is moved in a definite direction leading to the changes in length of the muscle filaments (sarcomere length) (Fig. 1b). One can regard this sliding filament concept as a paradigm in biological sciences as Thomas Kuhn has defined.

This article is mainly concerned with the events leading to the simultaneous publication of the two *Nature* papers. Attention will be especially directed to the roles played by both Huxleys and Hanson, and to their mutual relationship at that time.

The Huxley-Niedergerke work

Andrew Fielding Huxley, born at Hampstead, London, in 1917, was educated as a physiologist at Trinity College, Cambridge, and was an assistant director of research at the Department of Physiology, Cambridge, from 1951 to 1959. In 1951 he changed his research subject from nerve to muscle after completing the monumental papers with Alan Hodgkin on the excitation mechanism of squid giant axon (Nobel Prize for Physiology or Medicine, 1963) (*10, 11*). Huxley had become interested in the structure and function of striated muscle through the stimulating lectures of David Hill, a muscle physiologist [son of a great muscle physiologist, Archibald V. Hill (1886-1977), Nobel laureate (1922)], while he was asked to take over Hill's lectures in 1948 (*10*).

Huxley started his muscle work by developing a new interference microscope suitable for the observation of striation changes during muscle contraction. Assisted by the firm of R. & J. Beck, he undertook to construct a high-power interference microscope, and his hand-made microscope was successfully in use (*12*) by the end of 1952 (*10*).

In the autumn of 1952, Rolf Niedergerke, born at Mülheim-Ruhr, Germany, in 1921, joined Huxley's laboratory (*13*). He had worked on isolated nerve fibers in Alexander von Muralt's Institute in Berne. Robert Stämpfli, who taught Niedergerke nerve fiber dissection, recommended him to Huxley as a collaborator for isolation of single muscle fibers (*13*). Niedergerke had been a demonstrator in physiology at Göttingen (*13*).

Huxley and Niedergerke quickly reached a preliminary conclusion that the A band width in a sarcomere was fairly constant during passive stretch, isometric contraction and also isotonic contraction, to a certain extent. The force was generated while the A band remainded invariant during isometric contraction. These results were obtained by early 1953 (*11*). However, completion of the work was delayed due to Huxley's duties as the editor of the *Journal of Physiology* and the secretary of the Council of Trinity College (*11*).

Niedergerke, who had read a number of papers on the myofibrillar structure by 19th century German workers, pointed out to Huxley the idea by Wilhelm Krause (1869) (*14*) that the A band (width, 1.5 μm) in mammalian muscle consisted of longitudinally parallel rodlets (filaments) and the rodlets did not change in length during contraction, but attracted "fluid" from the adjacent I bands. This is a prototype of the sliding concept, if "fluid" is read as

"filaments" distinct from the A band rodlets. Huxley carefully examined the microscopists' work and later wrote historical overviews (*10, 15*).

A cine film taken in March 1953 first suggested to Huxley a sliding filament system (*10*). A single fiber, contracting in response to a slowly increasing current, showed the formation of the first contraction band as a narrow dense line at the middle of the A band. Huxley assumed that the A band consisted of one kind of filaments as Krause had already mentioned (*14*) and further that there were another kind of filaments in the I band. The latter I filaments would be separated in the A band in a sarcomere at rest length. It followed that the first contraction band would be due to the collision between opposing sets of these I filaments at the center of the A band. The second contraction band formed near the Z line on further contraction would be due to the collision of the A filaments with the Z line. Huxley had known of the presence of two kinds of muscle contractile proteins, myosin and actin, discovered and characterized by Albert Szent-Györgyi and his school at Szeged (*16*). Furthermore, Hugh Huxley had reported the presence of two kinds of longitudinal filaments in a sarcomere based on low-angle X-ray diffraction and electron microscopic photographs of rabbit psoas fibers (*17, 18*). He tentatively regarded the two kinds of filaments to be myosin, located in the A band, and actin, mainly located in the I band.

Although the main result of the Huxley-Niedergerke paper was the constancy of the A band width of isolated frog muscle fibers during contraction and relaxation, the insight into deeper understanding of the mechanism of muscle contraction is indeed penetrating (*1*): "... makes very attractive the hypothesis that during contraction the actin filaments are *drawn into* the A-bands, between the rodlets of myosin." "If a relative force between actin and myosin is generated at each of a series of points in the region of overlap in such sarcomere, then tension per filament should be proportional to the number of this zone of overlap" (*19*).

These implications, later proved to be actually the case, are most significant, making the Huxley-Niedergerke paper distinct from a mere observation that the A band width was constant during contraction and relaxation (*20*).

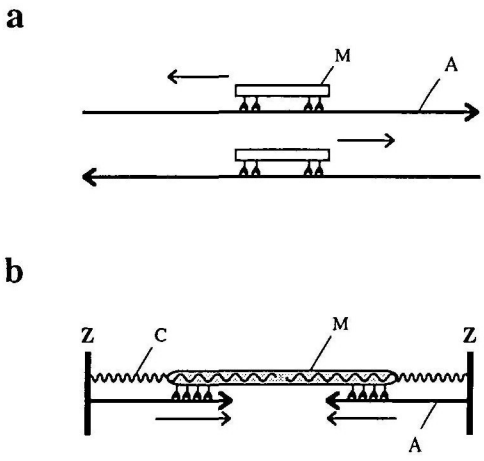

Fig. 1. **Sliding movements of myosin motor and actin rail.** a, actin rail is immobilized. Myosin motor moves on actin rail to the direction of barbed (plus) end of the actin filament. b, myosin motor is immobilized. Actin rail moves to the direction of the pointed (minus) end of the actin filament. This is the case with striated muscle contraction. M, myosin; A, actin; Z, Z line; C, connectin/titin. Modified from Hayashi and Maruyama (*9*). Courtesy of Yukiko Ohtani.

Hugh Huxley and Jean Hanson

Hugh Esmor Huxley, born at Birkenhead, Cheshire, in 1924, was educated as a physicist at Christ's College, Cambridge. H.E. Huxley (HEH) is not related to A.F. Huxley (AFH) (*21*). HEH became interested in biophysics rather than nuclear physics. After having spent 4 years as a research student at the MRC unit of Molecular Biology at Cavendish Laboratory, working first on crystalline proteins and then on muscle, he went to Francis O. Schmitt's (1903-) laboratory at the Massachusetts Institute of Technology (MIT), Cambridge, MA, as a Commonwealth Foundation fellow from 1952 to 1954 to continue his work on muscle.

Fig. 2. **A.F. Huxley and H.E. Huxley.** Near Mt. Fuji, 1979. Courtesy of Mrs. Fumiko Ebashi.

Fig. 3. **Jean Hanson.** King's College Archives. Courtesy of Dr. Pauline Bennett.

Contact with MIT was established through John Kendrew, his supervisor (*22*).

When HEH was engaged in the X-ray diffraction work on myoglobin under Kendrew's guidance, he read a number of papers on biological structures. He happened to read an X-ray diffraction paper on dried muscle by Bear (*23*). Electron microscopy showed that myofibrils were mainly composed of longitudinal "actomyosin" filaments (*24*). This pioneering work on muscle structure was carried out in Schmitt's laboratory at MIT. HEH became interested in the fine structure of "hydrated" (not dried) muscle and decided to do low-angle X-ray work using a hand-made X-ray camera suitable for the detection of 300-400 Å spacing. Living frog muscle gave two sharp equatorial X-ray diffractions, *i.e.* in a direction transverse to the fiber axis showing a hexagonal array of very long filaments 450 Å apart. Surprisingly, the pattern of glycerinated rabbit psoas fibers (rigor state) was considerably different: the hexagonal array of the filaments 450 Å apart did not change, but the intensity at 450 Å spacing (1,0 reflection) became much weaker and that at the 225 Å spacing (1,1 reflection) became much stronger. HEH interpreted these changes as follows: there were two sets of hexagonal arrays, myosin (primary) and actin (secondary). Szent-Györgyi's school had shown that there were two structural proteins involved in the contractile material of a muscle, actin and myosin (*16*). In rigor muscle, actin attached to the outside of the myosin by specific linkages, producing a high electron density in a specific region in between the myosins (*25*). Furthermore, if the ATP-containing muscle is stretched by up to 40%, then the axial pattern remained unchanged.

There is an anecdote involving Hugh Huxley and Dorothy Hodgkin (1910-1994; Nobel Prize for Chemistry, 1964). According to the late Sir John Randall (*26*), at the Ph.D. examination for HEH in 1952, Hodgkin, one of the examiners, put forward a kind of sliding-filament mechanism to account for HEH's X-ray diffraction work. However, what Hodgkin mentioned was related to the sliding movement of actin filaments to associate with myosin filaments that actually did not occur in rigor (*26, 27*). She thought that when a muscle went into rigor, it shortened. HEH strongly and successfully resisted her suggestion that the changes on the 1.0/1.1 ratio that he saw when the muscle went into rigor had anything to do with changes in muscle length (*28*).

On September 2, 1952, HEH arrived at the Department of Biology, MIT. He wanted to learn electron microscopy, to reveal the presence and localization of myosin and actin filaments in a myofibril (*27*). Francis O. Schmitt's laboratory was world famous for elucidating the fine structure of collagen fibrils and many other biological structures (*22*). Alan Hodge and David Spiro were working on muscle structure under an electron microscope. Hodge gave HEH some helpful technical instructions into the operation of the electron microscope. Hodge, born in Singapore in 1926, graduated from the University of Western Australia and got his Ph.D. on the study of paramyosin from MIT in 1952 under F.O. Schmitt's supervision. In early 1953 Hodge returned to Australia and continued his muscle and other research at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Melbourne (*29*).

Jean Hanson, born at Newhall, Derbyshire, in 1919, graduated from Bedford College, majoring in Zoology in 1941 (*26, 30*). As a research student at the Department of Zoology, Bedford College, Hanson did some histological work on the annelid vascular system and became interested in obliquely striated muscle (*31*). In 1947 she joined the MRC unit of Biophysics directed by John Randall (1905-1984) at King's College. In this unit, Rosalind Franklin (1920-1958) first obtained X-ray diffraction evidence, eventually leading to the double-helical structure of DNA proposed by Watson and Crick.

After an investigation of the fine structure of snail spermatozoa in collaboration with Randall (*32*) in 1951, Hanson began to observe structural changes of isolated myofibrils during contraction under a phase-contrast microscope (*26*). It was observed that glycerinated rabbit myofibrils in the presence of ATP contracted slowly to about 57% of the original length and on further shortening second contraction bands were formed at the Z line positions (*33*).

For further structural investigation she hoped to refine her technique of electron microscopy in Schmitt's laboratory at MIT. Randall recommended her to Schmitt (*26*). With a Rockefeller Foundation fellowship Hanson went to MIT in February 1953 (*26*). On her arrival Hugh Huxley had already started electron microscopical work with muscle. He had obtained electron micrographs of cross-sections of muscle showing the double array of filaments, but at that time he had not realized that it only occurred in the A band overlap region (*34*). After active discussion with HEH, Hanson decided to collaborate with HEH by extending her light microscope work at King's College (*26*). From time to time, however, she improved her electron microscopical technique (*22*) that would contribute to her later muscle research.

Huxley-Hanson work

Before describing the 1954 *Nature* paper, it is worth briefly mentioning two articles by H.E. Huxley and Hanson published in 1953. These studies were carried out at MIT. It should be mentioned here that Schmitt was generous to Huxley and Hanson over their publications, and did not put his name on their papers.

In collaboration with Hodge and Spiro, a new type of ultramicrotome was developed in 1952 (*35*). Using this apparatus, HEH examined the cross-sections of rabbit and frog skeletal muscles to locate myosin and actin filaments (*18*).

In the I band, disorganized small (thin) filaments of 50 Å in diameter were observed, whereas in the H band of the A band, hexagonal arrays of large (thick) filaments of 110 Å in diameter 200-300 Å apart were present. In the A band, except for the H band, there were two kinds of hexagonal arrays, thick and thin filaments. In cross sections, the thin filaments lay at points equidistant from three thick filaments around it. Thus HEH's interpretation of his low-angle X-ray diffraction work was largely supported by electron microscopic examinations. The discrepancy between the X-ray spacing of 450 Å and the electron microscopy value of 200-300 Å might be due to lateral shrinkage during the EM preparation procedure, fixation, dehydration and embedding. It is of interest to note here that the word of "sliding" was used for the first time in this article (*36*) in the context of the extensibility of muscle: "the two sets of filaments slide past each other." This was because glycerinated myofibrils (in rigor) were completely inexten-

sible, but became extensible on addition of MgPP or a high concentration of ATP. The latter was due to dissociation of actin from myosin.

Hanson and HEH jointly carried out light microscope study (*36*). Hanson had become familiar with biochemistry of muscle proteins. When myofibrils were treated with myosin-extracting solution (0.3 M KCl, 0.15 M phosphate buffer, pH 6.5, and 0.4 mM ATP or 0.47 M KCl, 0.1 M phosphate buffer, pH 6.4, 10 mM PP, and 1 mM $MgCl_2$), the A bands largely disappeared. Longitudinal sections of such muscle fibers revealed that the thick filaments at the A band almost disappeared leaving thin filaments probably extending from the original I band region. Thus it was logically concluded that the thick filaments at the A band were composed of myosin. This in turn strongly supported the view that the thin filaments consisted of actin. It is to be pointed out that the 1953 *Nature* paper (*36*) was really a major milestone, because it established the fine structure of striated muscle, with actin and myosin in two sets of separate but partially overlapping filaments.

In the 1954 *Nature* paper (*2*), HEH and Hanson summarized microscopic observations of isolated rabbit muscle myofibrils reinforced by electron microscopic examinations of the muscle fibers.

First, the constancy of the A band length was observed during contraction up to about 65% of the rest length of sarcomeres. The changes in the sarcomere length were taken up by changes in the I band length alone. This was in good agreement with the Huxley-Niedergerke work on intact muscle fibers (*1*). Using a myofibril one end of which attached to cover slip and the other end of which attached to slide glass, it was possible to manipulate both isometric and isotonic contraction. The constancy of the A band length was also confirmed during isometric contraction. When stretched in the presence of a large amount of ATP (10 mM), both I band length and H band width increased. HEH and Hanson also noted that the closing up of the H zone region at constant A band length during contraction, and the corresponding closing up of the H zone gap between the ends of the I bands seen after myosin extraction of contracted myofibrils.

In the previous work (*36*) the extraction of the A band was incomplete. Hasselbach reported that it took some time to remove myosin entirely to give rise to A band-free sarcomeres (*37*). HEH and Hanson obtained the latter by extraction with 0.1 M PP and 1 mM $MgCl_2$. The A band-free myofibrils did not contract at all in the presence of added ATP. Electron microscopy confirmed the absence of the thick filaments at the original A band region leaving thin filaments extending from both Z lines in a sarcomere. The continuity and elasticity were maintained in the A band-free sarcomeres. Therefore, HEH and Hanson assumed that an elastic filament called the S filament linked opposing pairs of thin filaments from both Z lines in a sarcomere (*38*). "S" was taken from stretch (*34*).

It is quite clear that this Huxley-Hanson paper presented strong evidence for sliding movement during muscle contraction and relaxation, as described: ". . . actin filaments slide out of or into the parallel array of myosin filaments in the A bands." A full account was published in 1955 (*39*).

Why were the two 1954 papers printed jointly?

A.F. Huxley stayed at the Marine Biological Laboratory, Woods Hole, MA, in the summer of 1953 (*10*). There he met Hans H. Weber, a leading German muscle physiologist (*40*). Weber told AFH that Wilhelm Hasselbach, his collaborator, had been able to identify myosin as the A band substance (*37*). Also, AFH met HEH at Woods Hole and they discussed their results. HEH told him his work about the double array of filaments in striated muscle. AFH learned that Jean Hanson and HEH also had observed A band-extracted myofibrils. In turn HEH was told about the constancy of the A band width of frog muscle fibers during contraction. AFH suggested to HEH that they should get in touch with each other on further progress of work of mutual interest (*26, 41*). AFH (*10*) acknowledged that HEH's paper (*18*) in 1953 contained the first proposal of the sliding filament concept.

In January or February 1954 (*28*), HEH was writing a joint paper with Hanson and wrote to AFH that HEH would like to cite AFH's paper if submitted. AFH suggested that they should publish the two papers together in *Nature* (*10, 11, 41*). Interestingly, both Schmitt (*22*) and Randall (*26*) recalled that each of them transmitted the Huxley-Hanson paper to *Nature*. The two manuscripts were duly published together in the same issue of *Nature* (May 22, 1954).

Response to the sliding filament concept

The sliding filament concept was not immediately accepted by most biologists. Albert Szent-Györgyi (1893-1986), father of ATP-actin-myosin research, was strongly against the two kinds of separated filaments system, when HEH personally communicated to him at Woods Hole in early 1953 (*28*). Szent-Györgyi had the view that "actomyosin" filaments run continuously through the sarcomere (*28*). However, he later admitted that he felt ashamed of himself that he had not thought of a sliding concept. Eventually, he decided to leave muscle research (*42*).

When Jean Hanson spoke at the Symposia of the Society for Experimental Biology held at Leeds in September 1954 (*39*), William Astbury and other physicists were all negative about the concept of the directional movement of filaments (*43*). Even in the early 1960s when a symposium of biomacromolecules was held in Pittsburgh, PA, most physical chemists including Paul Flory (1910-1985; Nobel Prize for Chemistry, 1974) strongly objected to the directionality of the filament movement. The writer still remembers Jean Hanson shouting: "I know I cannot explain the mechanism yet, but the sliding is a fact."

The sliding filament concept was gradually accepted by mid 1960s. Visualization of the sliding movement was first achieved in the field of cell biology (*44*): myosin-coated plastic microspheres moved in one direction on actin filaments oriented in the *Nitella* gel layer. It was demonstrated that the movement of single actin filaments (fluorescent-dye conjugated) on a slide covered with myosin (*45, 46*) and efforts are currently being made to elucidate how chemical energy of ATP is converted into mechanical energy and how unidirectional movement occurs, on the basis of the three dimensional structures of the myosin head and of actin (*47*).

Epilogue

In 1957 Andrew Huxley published a cross-bridge theory to explain the basis of filament sliding (*3*) that greatly contributed to the establishment of the sliding filament

theory. He extented his theory together with Robert Simmons in 1971 (*48*). The 1957 theory dealt only with the attachment and detachment of cross-bridges while the 1971 theory with Simmons dealt with what the cross-bridges might be doing while attached. He was the Jodrell Professor of Physiology, University College London from 1960 to 1969, then a Royal Society Research Professor (1969-1983). He served as President of the Royal Society (1980-1985) and was Master of Trinity College, Cambridge from 1984-1990. Sir Andrew Huxley, OM, still is active as a researcher at Cambridge.

Rolf Niedergerke went to the Department of Biophysics, University College London, in 1954, becoming a reader in 1963. He continued to work on muscle, with many distinguished contributions on the calcium activation of cardiac muscle (*13*). Since his retirement in 1986, he has been actively engaged in research as an emeritus reader there.

In April 1954 Hugh Huxley returned to England and extended his electron microscopic work at Cambridge and University College London (1956-1961). He published an elegant electron microscopic study of muscle in 1957 (*4*) and furthermore, showed polarity of the location of cross-bridges on the myosin filament as well as of the actin filaments in a sarcomere (*49*). He continued his X-ray work on muscle contraction at the MRC Laboratory of Molecular Biology, Cambridge from 1961-1987 and received a Royal Medal from the Royal Society in 1977. In 1988 he moved to Brandeis University, MA, as professor of the Rosenstiel Medical Sciences Research Center. He served as the director there (1988-1994).

Jean Hanson continued her muscle research at King's College after her return from MIT. In 1963 she demonstrated double stranded structure of the actin filament with Jack Lowy (*50*). She was promoted to professor in 1966 and became the director of the Muscle Biophysics unit, King's College in 1970. In August 1973 she suddenly died of fulminating meningococcal septicaemia of the adrenal cortex (Waterhouse-Friederichsen Syndrome) (*26, 51*). Her strong enthusiasm for science and warm personality will be long remembered by those who knew her.

The writer is most grateful to Sir Andrew Huxley and Professor Hugh Huxley for their helpful personal communication. He is greatly indebted to Professor John T. Edsall, the writer's supervisor in the history of science, for his warmest encouragements and reading through the manuscript. Thanks are due to Professors F.O. Schmitt, J. Lowy, A.J. Hodge, R.M. Simmons, S. Ebashi, and M. Endo for their invaluable information. He is especially indebted to Professors Simmons and Hodge and Dr. R.T. Tregear for their scrutinizing the manuscript. Thanks are also due to Professors M.F. Morales, J. Gergely, and H. Noda for their helpful comments.

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min in 1899, never got a Ph.D., and was just Mr. Hardy, not Professor or Doctor, until he was knighted and became Sir William. That happened early in 1925, and F.G. Hopkins got a knighthood at the same time. I remember that very well, because I had started working in the Hopkins Laboratory in Cambridge, just a few months before that. People of their generation in England, I think, never took Ph.D.s."
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