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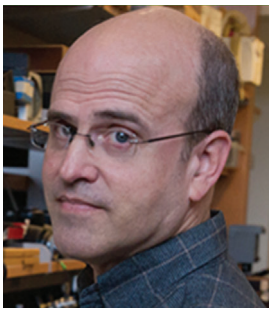
History



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**Abstract** Explanations for protoplasmic streaming began with appeals to contraction in the eighteenth century and ended with appeals to contraction in the twentieth. During the intervening years, biologists proposed a diverse array of mechanisms for streaming motions. This paper focuses on the re-emergence of contraction among the molecular mechanisms proposed for protoplasmic streaming during the twentieth century. The revival of contraction is a result of a broader transition from1 colloidal chemistry to a macromolecular approach to the chemistry of proteins, the recognition of the phenomena of shuttle streaming and the pulse of protoplasm, and the influential analogy between protoplasmic streaming and muscle contraction.

**Keywords:** Actin; cytoplasmic streaming; history of cell biology; protoplasm

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## INTRODUCTION

In November of 1940, *Time* magazine celebrated the "primordial heartbeat" manifest in the "pulse of protoplasm" from the lowly slime mold, *Physarum polycephalum* (Anonymous 1940). The pulses and rhythms of protoplasmic or cytoplasmic streaming were not new in 1940. The "circulation of cell sap" had been described as early as 1774 by Bonaventura Corti, and in the intervening years protoplasm had been elevated to become the foundation of life by Thomas Henry Huxley and Ernst Haeckel, with one of its most important properties being its streaming motion (Corti 1774; Huxley 1869; Haeckel 1869; Geison 1969). Indeed, by the mid-twentieth century, theories explaining the motion of protoplasm were plentiful in the scientific literature (Seifriz 1943). Even the pulse of *Physarum* had graced the pages of *Time* only 3 years earlier in a feature describing Professor William Seifriz's trip to the Pasteur Institute to film the elusive pulsing movement (Anonymous 1937). What brought the pulse of the protoplasm back to the pages of *Time* in 1940 was a new set of imaginative experiments for its manipulation, conducted in William Seifriz's University of Pennsylvania laboratory by the young Japanese researcher, Noburo Kamiya.

Born in 1913, Kamiya had graduated from the University of Tokyo in Japan and was studying in Germany at the Botanical Institute of the University of Giessen with Professor Ernst Küster when, in 1939, he was told by the Japanese Embassy that he should leave immediately. With 180 other Japanese citizens, Kamiya sailed on the *M. S. Yasukunimaru* for Yokahama via Bergen, Norway, New York, and San Francisco. Germany invaded Poland as Kamiya was in transit. With research in Germany out of the question, he set his sights on the United States and began to learn English on the transatlantic voyage. Kamiya had heard of William Seifriz in Germany, and arranged for an introduction through officials at the Japan Institute in New York. Fortunately for Kamiya, Seifriz spoke fluent German, which gave them a common language. More importantly, Kamiya had just published a short paper on the rhythmic movement of euglenoids that fit beautifully with Seifriz's passion for biological pulses and rhythms. Seifriz graciously accepted Kamiya as a refugee student into his laboratory and arranged for his support (Kamiya 1989).

At Penn, Kamiya was introduced to protoplasm research using Seifriz's cultures of *Physarum.* Kamiya adopted Seifriz's physical and chemical approach, including microdissection. As he set about experimenting on factors that would alter the flow of protoplasm, Kamiya noticed that mechanical pressure changed the speed and direction of streaming. At the time, a variety of chemical, electrical, and physical forces had been invoked to explain protoplasmic streaming (more on this below), but pressure was relatively unexplored. Kamiya's real innovation, however, lay in his double chamber method, which he used to measure and manipulate pressure and streaming. Kamiya discovered that he could stretch a blob of protoplasm so that it remained connected by a thin thread suspended in warm agar. Placing each blob in a separate

airtight container, he could adjust the air pressure in each chamber, and in doing so stop, start, and reverse the direction of flow in the connecting thread. Air pressure in the double chamber method was translated into motive force and a new method for measuring and quantifying streaming was born. What excited Seifriz most, however, was that the pulses in the protoplasmic stream could be charted (Kamiya 1989). As never before, the rhythms of protoplasmic streaming could be represented as waves, just as the ebb and flow of blood in our arteries can be represented on an EKG chart (Figure 1). These traces graced the pages of *Time* in 1940, and celebrated this international collaboration in the days before Pearl Harbor (Anonymous 1940).

As thrilled as Kamiya and Seifriz were about this new representation of the "pulse of protoplasm", they were far from explaining why it was there. In fact, what they had done was to introduce and bolster a new aspect of the phenomena of streaming. The challenge that lay ahead was that of finding mechanisms that could explain this pulsing motion as well as other forms of motion such as the jumpy saltational movements of particles within a cell and the rotational movement of the protoplasm.

Seifriz and Kamiya introduced the pulse of protoplasm at a time when studies of protoplasm and its motion were shifting from an approach dominated by colloidal chemistry to an approach that concentrated on the macromolecular features of proteins. New research by William Astbury on the structures of fiber proteins and advances in the study of muscle contraction after the Second World War led Seifriz and others to revisit an old idea that posited contraction as the means for producing protoplasmic motion. Rhythmic contraction also had the advantage of explaining the pulsing movement of protoplasm in terms of repeating cycles of contraction and relaxation. What distinguishes post-war contraction theories from those of Corti and other earlier scientists is the macromolecular approach of later scientists that led them to search for the basis of cellular motion in the structural properties of large protein molecules. While advances in visualization allowed biologists to see new fibrous structures within the cell, the macromolecular approach lead them to characterize those structures in terms of their constituent components, e.g., actin and myosin in the case of streaming.

## WILLIAM SEIFRIZ AND PROTOPLASM AT PENN

William Seifriz (Figure 2) was a self-described "protoplasmatologist." Born in Washington DC in 1888, Seifriz earned both is B.S. and Ph.D. at Johns Hopkins University. Doctorate in hand, he left the US in 1920, taking the long way to Switzerland via Japan and India. A 6-month stay in the laboratory of Robert Chodat in Geneva reinforced his interest in both plant biogeography and the physiology of algae (Rendle 1934). As a graduate student, Seifriz used microdissection to analyze the viscosity of protoplasm in a variety of organisms (Seifriz 1920). His interest in protoplasm's properties as a colloidal solution lead him next to England to study colloid chemistry, followed by a longer stay in Berlin at Herbert Freundlich's laboratory at the Kaiser Wilhelm Institute for Physical Chemistry and Electrochemistry (Kamiya 1956). Freundlich was a well-known colloid chemist with an interest in applying colloid science to research on protoplasm (Donnan 1942). Colloids were understood to be permanent suspensions of one kind of microscopic matter in another. Freundlich and others took a physical and chemical approach to the properties of colloids examining, for instance, their viscosity, elasticity, and reactivity as emulsions and gels (Deichmann 2007). Seifriz formed a lasting relationship with Freundlich, and became a leading advocate for understanding protoplasm as a colloidal system (Freundlich and Seifriz 1923; Seifriz 1936).

When he returned from Germany, Seifriz continued his work on protoplasm from the perspective of colloidal

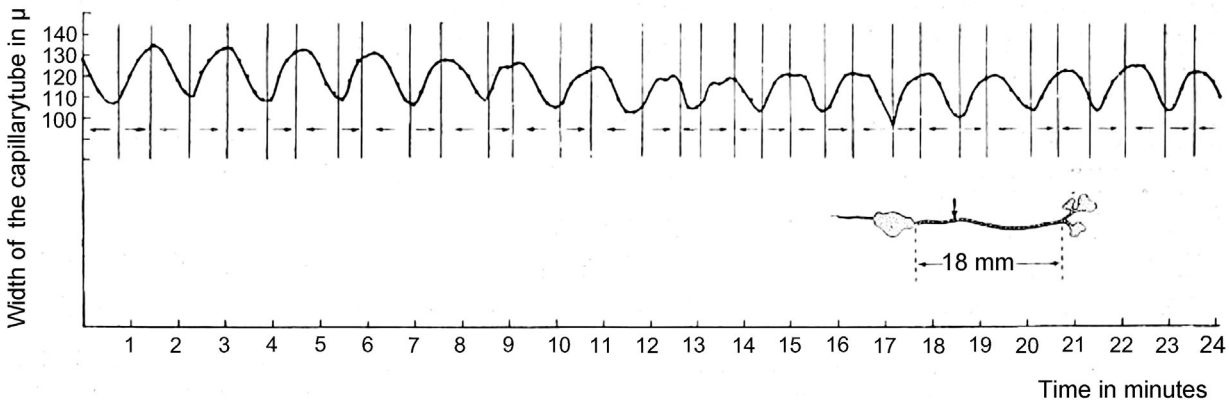


**Figure 1. Kamiya's measurement of the pulse of protoplasm in the capillary tube of Physarum**
Kamiya measured the flow of protoplasm at a spot of the capillary tube of Physarum (indicated by the arrow pointed at the cell on the right side of the figure) by measuring the width of the capillary tube at the region every ten seconds. The rhythmic changes correspond to the shuttle motion of the pulse of protoplasm and confirm measurement taken volumetrically using Kamiya's double chamber method (Kamiya 1950).

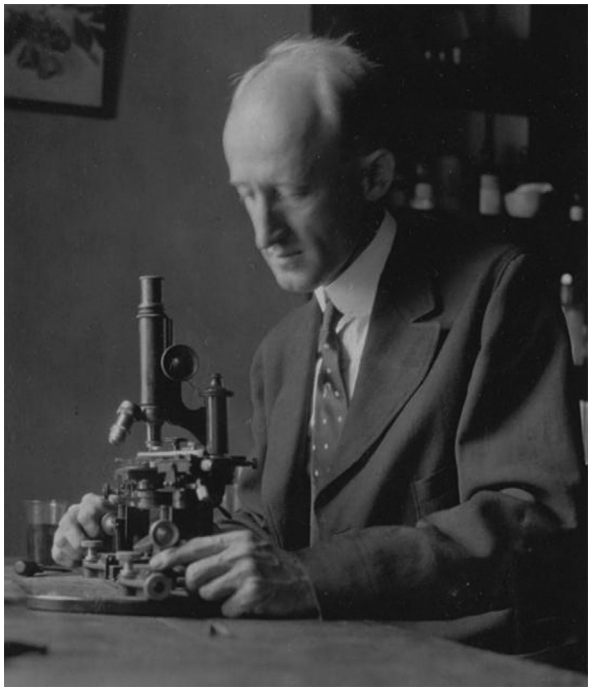

**Figure 2. William Seifriz at his microscope in 1927**
Image from Unknown (1927).

chemistry, first at Yale and then the University of Michigan, before he became a National Research Fellow at the University of Pennsylvania, where he was appointed to the faculty in 1925 (Kamiya 1956). At Penn, Seifriz pursued his interests in protoplasm and plant biogeography; publishing an extensive array of papers on both, as well as a series of papers just on the chemical properties of emulsions (see the publication list in Kamiya 1956). While his early work on protoplasm concentrated on structural features, by 1937 he began to widen his research to consider the problem of explaining motion (Seifriz 1937). In particular, Seifriz became fascinated by the "shuttling motion" in filaments that described flow in one direction that then reversed. Not satisfied that changes in hydration and water flow could explain this change of direction, Seifriz began his own cinematic investigations of the ebb and flow of protoplasm (Seifriz 1937). However, his own films paled in comparison to those being produced at the Pasteur Institute by Jean Comondon, so Seifriz arranged to visit Paris to film shuttling motion with high temporal resolution (Anonymous 1937). And, there, thanks to the then extraordinarily high frame rate of Comondon's equipment, Seifriz measured a regular cycle of forward flow and its reversal every 40 or 45 seconds. Seifriz hypothesized that this pulse was the result of a rhythm of contraction and expansion of the protoplasm, and even suggested that it might analogous to the "rhythmical contraction of sympathetically controlled muscle tissue" (Seifriz 1937, p. 398).

At the time, protoplasm enjoyed special status in biology. Huxley, Haeckel, and many others viewed it as the fundamental stuff of life. Investigating its properties, especially its motion, was akin to searching for the basis of life itself. Descriptions of protoplasm, analyses of its structure, and theories for its motion abounded in the scientific literature. Seifriz tried his hand at systematizing this knowledge in a lengthy article for *The Botanical Review* published in 1943 (Seifriz 1943).

In "Protoplasmic Streaming", Seifriz distinguished streaming, as flow of protoplasm within a cell, from amoeboid movement and euglenoid movement, which both changed the overall shape of the cell body. He also did not want to confuse streaming with organelles that could move independently within the cell; streaming had to be a feature of the protoplasm itself. Although he offered a classification of movement that featured five categories (agitation, rotation, circulation, shuttle, and sleeve), his interest was on the organized directional movement seen in the rotational flow at the periphery of cells in *Nitella* and the characteristic ebb and flow of shuttle movement he observed in his slime molds. With characteristic care, he reviewed 11 proposed mechanisms for these various kinds of motion before championing contraction as the cause of protoplasmic streaming.

The evaluation of mechanisms of protoplasmic streaming by Seifriz and others frequently depended as much on the constitution of the phenomena as the nature of the proposed mechanism. Appeals to hydration and osmosis, for instance, were both discussed as possible causes of directional flow, but ultimately rejected by Seifriz because they could not explain the complex multidirectional flows observed in circulation or the reversals seen in shuttle motion. Physical theories based on surface tension, electricity, and magnetism were all discussed as proposed mechanisms that corresponded to cellular properties. However, Seifriz noted that demonstrating that a cell exhibited surface tension or responded to an electrical potential difference were not sufficient to demonstrate that protoplasmic streaming was caused by these finds of physical phenomena. Electrical potentials were present, and they did effect protoplasm, but how they did so, and whether they had enough influence to create the different forms of streaming had not been demonstrated.

A different kind of explanation was represented by the extension of sol-gel reversibility from amoeboid movement to protoplasmic movement. Samuel O. Mast at Johns Hopkins University had proposed that amoebae move in part because of the flow of protoplasmic solution that reacts to become a gel surrounding the flowing boundary of solution (Mast 1923). Seifriz was unconvinced. Protoplasmic flow in *Amoeba* was understood to be unidirectional, and he did not see a way for sol-gel reversibility to produce the kind of reversals and rhythmicity observed in the shuttle streaming of plant and fungal cells.

The only way that Seifriz saw to explain the pulse of protoplasm was to return to theories of contraction. In his review, Seifriz noted that contractility had been proposed by Corti in 1774 when he first described streaming motion in plants, and had enjoyed a long history since (Seifriz 1943, p. 110). Indeed, Otto Bütschli in his 1894 monograph on protoplasm notes that contractility had great appeal from the first observations of streaming because of the familiarity of muscle contraction and the desire to find "fundamental

properties" that can explain cellular motions (Bütschli 1894, p. 267). Nineteenth century advocates of the contractile theory of streaming proposed a "fibrillar reticular framework" that contracted and so drove the motion of the cellular matrix (Bütschli, p. 267). Similar fibers had been invoked by Theodor Boveri to explain the action of asters apparent in cell division, and Boveri had specifically suggested that asters might act just as muscle fibers do in contraction (Bütschli, p. 261). For Bütschli and others, however, the contraction theory was underdeveloped and could not explain why contraction in the amoeba seemed to occur at the forward edge of motion instead of the rear where it could drive motion forward (Bütschli, p. 270). In place of nineteenth century contraction theories based on fibrillar structures, many twentieth century biologists moved to explanations based on the chemical properties of solutions, such as the surface tension and sol-gel reversibility explanations mentioned above.

Seifriz's enthusiasm for the pulse of the protoplasm brought him back to a contractile mechanism whose cycles of contraction and relaxation could explain the rhythms that he and Kamiya had been able to quantify. The actual mechanism that Seifriz proposed was molecular, and based in the contractile properties of polypeptides. As he put it, "The contractility of protoplasm is due to the supercontraction of its principal structural proteins through the folding of molecular fibers symmetrically aligned and joined one to the other by side chains so as to form a three-dimensional lattice" (Seifriz 1943, p. 112).

The model for such polypeptides was the woolen fibers that the British physicist William Astbury had been analyzing using x-ray diffraction. In 1933, Astbury's *Fundamentals of Fibre Structure* had proposed that the elasticity of wool and hair fibers was a result of the molecular structure (Hall 2014). In 1938, Seifriz began to speculate that "contraction of folded molecules such as those postulated by Astbury for wool and hair" could form the molecular mechanism for streaming (Seifriz 1938, p. 25).

Astbury's study of protein structure did not end with wool. With a grant from the Rockefeller Foundation in 1938, he turned to the molecular components of muscle, and the molecule myosin in particular (Hall 2014, p. 80). Astbury was interested to see if myosin, as a fibrous protein, played a role in contraction by having the kind of folded molecular structure that he had found in wool. In 1940, he and Sylvia Dickinson reported that the keratin molecules in wool and the myosin molecules in muscle had important similarities in their structure, and that their transformation from folded to extended forms could underlie their elasticity (Astbury and Dickinson 1940). If Seifriz had been a fan of Astbury's work on wool, he was an even bigger fan of his work on myosin, and took Astbury and Dickinson's work on myosin as evidence that muscular contraction was based on the "supercontraction of the oriented myosin" (Seifriz 1942, p. 262). Seifriz did not claim that myosin was the protein behind protoplasmic contraction, but he was sure that a similar molecular mechanism was there to be found.

While Seifriz was assimilating Astbury and Dickinson's ideas on myosin, he accepted another refugee student, Ariel G. Loewy. Born in Romania in 1925, Loewy's family fled the rising political tensions in Europe in 1937 (Freeman 1996). They lived in England until 1941 before relocating again to Montreal, where Loewy attended McGill University. When Loewy applied to Penn for graduate school, he received a wonderful letter inviting him to join the Seifriz lab, and soon found himself researching the mechanisms of protoplasmic streaming. Loewy was given the task of elaborating the molecular mechanism that Seifriz had proposed earlier.

Loewy's work grew from that of Astbury and Dickinson, but in the late 1940 s incorporated new results from Albert Szent-Györgyi, the Hungarian chemist who had won the Nobel Prize in 1937 for his discovery of vitamin C. During the war, Szent-Györgyi had been working on myosin at the Institute of Medical Chemistry at the University of Szeged in Hungary. Although isolated from other scientists around the world during the fighting, Szent-Györgyi's work became widely known in the mid-1940 s (Szent-Györgyi 2004). Szent-Györgyi's group had differentiated two proteins involved in muscle contraction, myosin and actin (Szent-Györgyi 1942). In a pioneering series of experiments, his group then demonstrated that the interaction of myosin and actin in the presence of ATP mimicked the action of muscle contraction (Szent-Györgyi 1949).

Building on the work of Astbury's and Szent-Györgyi's groups, Loewy proposed a more detailed molecular mechanism for protoplasmic streaming, but, like Seifriz, stopped short of definitively demonstrating that myosin and actin were involved in plant protoplasmic motion. Loewy's idea was that contractile proteins created the motive force for streaming by being anchored on the edges of streams parallel to the direction of flow (Figure 3). Contraction and expansion of these fibrous proteins in phase with each other would create motion in the surrounding protoplasm (Loewy 1949). Loewy could not isolate the proteins that might be creating this motion, but he could determine whether or not they

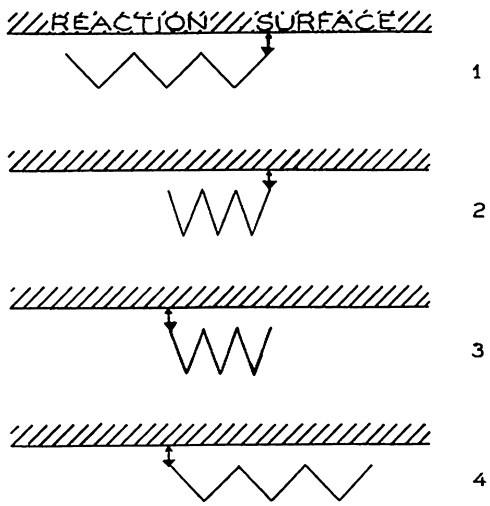


**Figure 3. Loewy's proposed molecular mechanism for protoplasmic streaming**
Extended (1 and 4) and contracted (2 and 3) forms of a protein molecule with different attachment points to the cellular substrate. Motion is created by the contraction and expansion of many molecules in phase with each other (Loewy 1949).

reacted to ATP in the same way as the myosin-actin complex in muscle. In a series of experiments published in 1952, Loewy demonstrated that molecules in *Physarum polycephalum* responded similarly to the muscle proteins described by Szent-Györgi's group (Loewy 1952).

For researchers like Kamiya, Loewy's results and similar findings from his colleagues Hiromichi Nakajima and Shigemi Abe in Osaka suggested that there were contractile proteins present in streaming cells that were similar to actin and myosin (Kamiya et al. 1957; Kamiya 1960). Loewy himself left streaming research convinced that he needed to know more about proteins. After earning his doctorate with Seifriz, he went to Harvard to learn how to fractionate proteins from blood, and, over the course of his career, elaborated and isolated many of the proteins involved in the blood clotting cascade (Freeman 1996).

Independently of Loewy, Albert Frey-Wyssling in Zürich, R. J. Goldacre and I. Joan Lorch in London, and Kurt Meyer and Herman Mark in Geneva and New York developed theories of streaming motion based on the folding and unfolding action of protein molecules (Frey-Wyssling 1949; Goldacre and Lorch 1950 Meyer and Mark 1951). While Goldacre and Lorch built their theory on the work of Astbury and Szent-Györgyi, as Loewy had done, Meyer and Mark claim to have developed their theory in 1929 based on their research on the molecular structures of silk, tendon, and muscle. In 1953, just 2 years before his death, Seifriz reviewed these theories and surprisingly found them wanting. Just as he had with earlier mechanisms of streaming, he worried that the "curling and uncurling of protein molecules" could not account for the range of motions associated with streaming or their locations within the cell. In their place, he postulated ionic impulses thought to travel through nerve and muscle fibers (Seifriz 1953). Despite Seifriz's skepticism, the molecular action of fibers, especially myosin and actin, could not be so easily dismissed from the literature on streaming motion.

## R. D. ALLEN, PRIMITIVE MOTILE SYSTEMS, AND THE AGE OF ACTIN

William Seifriz was not the only biologist at Penn taking a colloidal approach to protoplasm in the early twentieth century. Lewis Heilbrunn had been hired there in 1929. He had studied at Cornell University and then earned his Ph.D. with Frank R. Lillie at the University of Chicago in 1914 (Steinbach 1960). At Penn, Heilbrunn was appointed in zoology, and developed the general physiology program for premedical students and graduate students. In 1929, Heilbrunn was a recognized authority on protoplasm. Indeed, his book, *The Colloid Chemistry of Protoplasm*, had appeared the year before (Heilbrunn 1928).

Although Seifriz was appointed in the Botany Department at Penn, he and Heilbrunn took a very similar approach to the study of protoplasm. This is not to say that they agreed on how to study protoplasm or how to characterize it as a colloid. H. Burr Steinbach, Heilbrunn's student, describes Heilbrunn as "a man of strong opinions (who) had an ability to state them forcefully and clearly. His was a tough mind with little inclination to compromise" (Steinbach 1960). He did not compromise with Seifriz. In 1924, Seifriz used his microsurgical approach to produce an estimate of protoplasmic viscosity that was much higher than that obtained by Heilbrunn in 1914. Beginning in 1926, Heilbrunn issued what others perceived in the field as a "devastating critique" of Seifriz's method (Heilbrunn 1926, 1928; James 1937). While both continued to work on protoplasm, with Seifriz defending his microsurgical method, there is no evidence that Heilbrunn and Seifriz interacted at Penn despite their similar interests.

In the late 1940 s, Heilbrunn accepted Robert Day Allen into his lab as a graduate student. By that time, Heilbrunn was researching the effects of ions on cellular processes, especially the effects of calcium. Allen began studying cellular changes associated with fertilization in eggs of the clam, *Spisula solidissima*. In light of his later work on cellular movement, it is notable that even this early work included the tracking of granular movement within cells at the time of fertilization (Rebhun 1986).

As early as 1955 though, Allen's attention began to shift to the motion of amoebae (Allen 1955). Like many American cell biologists, Heilbrunn spent every summer at the Marine Biological Laboratory at Woods Hole, and took his graduate students with him. This is a habit that Allen continued. His first report on the motion of amoebae is a brief description of an experiment published in MBL's *Biological Bulletin*. In just a paragraph, Allen describes his study of amoebae in capillary tubes, which when broken, rupture the cell membranes and allow water to mix with protoplasm. The resulting protoplasmic streaming was cell free, but still contractile, which called into question Mast's cellular basis for contractile streaming. In his first full-length article on streaming, Allen and J. D. Roslansky from Princeton University used interference microscopy to measure a structural gradient within the amoeba.

In their conclusion, Allen and Roslansky critically surveyed mechanisms that could explain streaming. Their favored mechanism was a contractile mechanism based on the molecular folding and unfolding, as proposed by Goldacre and Lorch in 1950. The weakness of this theory and others, such as that based on the diffusion of water, was judged to be its inability to explain quick reversals in flow or opposing streams of protoplasm (Allen and Roslansky 1958). By 1961, however, Allen had supplanted his earlier support for Goldacre and Lorch with his own theory of amoebic movement, asserting that contractions did not occur in the tail but in the front of the amoeba, so instead of protoplasm being pushed, it was being pulled. These contractions were combined with differences in the constitution of the protoplasm that altered its viscosity and velocity (Allen 1961, 1962).

In the summer of 1962, Kamiya returned to the United States and worked at the MBL in Woods Hole before starting a year as a Visiting Professor at Princeton University, where Allen was an Assistant Professor. In Japan, Kamiya had been a lecturer in the Botany Department at the University of Tokyo, where he returned to his research on shuttle streaming in *Physarum* in 1945, and soon added experiments on rotational streaming in *Nitella* (Kamiya 1989). In 1949, Kamiya became a professor of cell physiology in the Department of Biology at Osaka University. He came back to work with Seifriz in the 1950s, and in 1960, a grant from the Rockefeller Foundation also allowed him to visit colleagues in the United States and

Germany. These trips allowed him to get visiting appointments in 1962 and 1963 (Kamiya 1978). During the early 1960s, Kamiya and Allen struck up a friendship and collaboration that lasted their entire lives. Their first joint effort was the organization of an international conference on "Primitive Motile Systems" held in April of 1963 at Princeton (Allen and Kamiya 1964).

The Primitive Motile Systems conference drew together 34 participants from the around the world to address basic systems of cellular motion, primarily motion within cells. The conference was later regarded as a watershed moment that revitalized the field of motility research (Rebhun 1986), and, I claim, spurred further research into the macromolecular basis of streaming motion. With Allen as an organizer, the nature of amoeboid motion was actively debated with over half of the papers focused on amoebae or amoeboid movement. Allen's advocacy of front-end contraction was confronted head-on by Goldacre, and discussed in great detail in the conference, happily recorded in the symposium volume under "free discussion".

An acknowledged starting place for the conference was the presence of contractile proteins that responded to ATP in a fashion similar to actin and myosin in muscle tissues. A number of papers discussed the microscopical evidence for cellular fibers, the molecular evidence that they suggested they are actin, and their possible role in cellular motility.

Fibrous proteins were the centerpieces of Robert Jarosch's presentation, as they had been the subjects of his research in the preceding decade. Jarosch was an Austrian microscopist, who worked in the Biological Research Division of the Austrian Nitrate Works before succeeding Ewald Schild at the Naturkundliche Station in Linz in 1962. In 1956, Jarosch had reported that protoplasmic drops extracted from *Chara foetida* contained fibrils that moved and formed polygons and loops (Jarosch 1958). That year, Kamiya and his student, Kiyoko Kuroda, replicated these results in Osaka with protoplasm from *Nitella flexis* (Kamiya and Kuroda 1956). Jarosch was a firm believer in the contraction theory of motion and following the work of Linus Pauling and Francis Crick sought to explain that motion in terms of the structures of helices, which he modeled as elastic screws. The rotation, pitch, and interaction of helices allowed Jarosch to postulate a number of molecular mechanisms for movement at a time when little was known about the molecular properties of observed cellular fibers. In his paper for the Primitive Motile Systems conference, Jarosch (1964) defended his elastic screw model, while Kuroda (1964) presented results from *Nitella* that suggested that the fibrils in isolated drops displayed "a remarkable undulating motion". This motion, however, was the result of many smaller fibers coming together to form larger structures, which suggested to Kuroda that streaming motion could be produced by these undulating fibers if they were organized in a cellular structure.

The search for fibrils was taken back to *Physarum* by Karl-Ernst Wohlfarth-Bottermann from the University of Bonn in Germany in his contribution to the conference (Wohlfarth-Bottermann 1964). Building on earlier work by his laboratory and others' who found "filamentous structures" in different species of amoeba, Wohlfart-Bottermann turned to *Physarum* because of biochemical work by Loewy, Kamiya, and others that suggested that there might be actin-like contractile proteins in these slime molds. Using electron and phase-contrast microscopy, Wohlfarth-Bottermann was able to visualize these fibrils, assess their responsiveness to ATP, and provide important cytochemical confirmation of Kamiya's biochemical studies of *Physarum*.

Despite the widespread interest in fibrils and contractility at the Primitive Motile Systems conference, in the end, the conference did not resolve the question of the nature of these molecular mechanisms as much as mark them as a focal point for further research. Consider the comments of Andrew Szent-Györgi in the closing discussion, where he said: "At this symposium there have been a number of references made and experiments presented which indicate that some of the filamentous structures and proteins may be similar to actomyosin, and that the motility of many cells may be based on a mechanism similar to contraction of muscle. I would like to point out that even if this analogy proves to be correct, this will not solve your problem." As Szent-Györgi and others at the conference knew all too well, it was not clear how the actomyosin system actually worked in the early 1960s. Hugh Huxley was developing the sliding theory of contraction, but Szent-Györgi suggested that motion would be easier to explain in terms of the action of polymers that converted chemical energy to mechanical work through structural changes (Szent-Györgi 1964, p. 626; Szent-Györgi 2004; Huxley 1969; Cooke 2004). Szent-Györgi's comments were prescient: actin-myosin mechanisms for streaming would take many years to establish, but, in the wake of the conference, the hunt for fibrils and actin in plant cells accelerated.

When Kamiya returned to Osaka, he directed one of his students, Eiji Kamitsubo to follow up on the work of Jarosch and Kuroda. At the time, Kamiya himself had been developing what he called the active shear theory for streaming that located the site of motive force at the sol-gel interface between stationary ectoplasm and moving endoplasm in *Nitella*. His work with Kuroda measuring velocity profiles supported this theory. The question remained, however, as to the mechanism that operated at this interface. Kamitsubo's research using centrifuged and intact cells of *Nitella* visually confirmed the presence of the fibrils detected by Jarosch and Kuroda, and implicated them in the production of streaming motion (Kamitsubo 1966a, 1966b). At the same time in Princeton, New Jersey, Reiko Nagai, working with Lionel Rebhun, also focused their attention on the ectoplasm-endoplasm interface. Building on Kamitsubo's research and the focus on fibrils from the 1964 conference, Nagai and Rebhun used an electron microscope to find bundles of microfilaments at this interface (Nagai and Rebhun 1966). For Nagai and Rebhun this visual evidence was the culmination of a body of research that suggested that these filaments were "the elements directly involved in developing this motive force" (Nagai and Rebhun 1966, p. 587). After spending time in Allen's laboratory as a visitor, Kamitsubo reinforced these results further by developing a method of dislodging chloroplasts to create a window in *Nitella* or *Chara* that made these fibrils visible with a light microscope (Kamitsubo 1972).

What was missing in the study of *Nitella* and *Chara* was confirmation that the now visible fibrils were actin. In 1963, Hugh Huxley had shown that heavy meromyosin forms a complex with actin with a visually distinct arrowhead pattern.

Harunori Ishikawa, Richard Bischoff, and Howard Holtzer extended this reaction beyond muscle cells in 1969 to show that many different kinds of cells that had not been previously thought to contain actin produced the characteristic arrowhead pattern when treated with heavy meromyosin (Ishikawa et al. 1969). A number of plant biologists tried to use heavy meromyosin to find actin in plants, but were unsuccessful until 1974 when two labs, one at Stanford University, and one in Cambridge, England, produced the telltale arrowheads.

Peter Hepler is a cell biologist who had earned a PhD at Wisconsin in 1964 before accepting a post-doctoral position with Keith Porter at Harvard in 1966 (Hepler et al. 2013). Hepler developed a program of research on plant microtubules under Porter and continued that research program as a faculty member at Stanford University. Hepler knew the literature on cytoplasmic streaming well, and knew many of the main contributors from Woods Hole (Hepler 2014). When his graduate student, Barry Palevitz, approached him about using heavy meromyosin to look for actin in *Nitella*, he remembers being skeptical that it would work, but agreed to let Palevitz give it a try (Hepler 2014). Just as it had in muscle cells, the actin in *Nitella* cells reacted with heavy meromyosin to form the arrowhead pattern discernible under the electron microscope (Palevitz et al. 1974; Palevitz and Hepler 1975). At roughly the same time, Richard Williamson was performing the same kind of experiment on the alga, *Chara corallina* (Williamson 1974). The consilience of the results left no doubt that plant cells contained actin at the site of cell motility.

But, remember Szent-Györgi's admonition at the Primitive Motile Systems conference, that finding actin is not enough to explain streaming. The research of Nina Allen in the early 1970s illustrates the challenge of explaining streaming, even after actin had been demonstrated in plant cells. Nina Strömgren Allen was the daughter of Danish astronomer, Bengt Strömgren, who worked for many years in the United States at the Yerkes Observatory (Allen 2014). She met Bob Allen when he spoke at the Smithsonian Institution in Washington DC, and in 1970 they were married. She finished her master's degree that year, and her doctorate in 1973. By that time, Bob Allen had moved from Princeton to SUNY Albany where he served as Chair of the Biology Department.

Using Kamitsubo's window technique with *Nitella* cells, Nina Allen was able to visualize the coordinated motion of fibers within the cells (Allen 1974). Based on these observations, she postulated a theory of motion where the undulating motion of microfilaments anchored in the ectoplasm generated motion in the endoplasm. Allen developed this theory in detail with careful calculations of structures, force, and velocity backed by careful microscopic observation. After the discovery of actin in *Nitella*, Bob and Nina Allen grappled with the issue of the role of actin in motility in their 1978 review of streaming in plants (Allen and Allen 1978). The evidence for fibrils and filaments was undeniable, but they were unclear whether the undulations of those filaments seen so clearly in *Nitella* were actively producing motion or passively being moved. Kamiya was not convinced by Nina Allen's proposal of undulating fibers driving protoplasmic streaming, because he felt it could not produce the kinds of velocity profiles that he observed and that formed the basis of his active shear theory (Kamiya 1981). Instead, influenced by research on sliding mechanisms in muscle, especially that of his Japanese colleague Setsuro Ebashi, Kamiya favored the idea that active shear and protoplasmic motion was produced by myosin in the protoplasm sliding along actin fibers (Kamiya 1986). Indeed, Kamitsubo had suggested in 1972 that myosin on cellular particles in the endoplasm attached to actin filaments anchored in the ectoplasm or cortex and slid along them (Kamitsubo 1972). In her review in 1980, Nina Allen considered this alternative, and admitted that if the undulating fibers were putative actin molecules, then it was possible that "networks of putative myosin (also lacking positive biochemical identification) move along the actin filaments" (Allen 1980, p. 795). In this view, direct actin-myosin interactions produces sliding in plant cells as it does in muscle. This would have the virtue of explaining the active shearing in rotational motion as well as fountain streaming and saltation by similar mechanisms, although the Allens admitted in 1978 "there is no evidence to confirm this idea" (Allen and Allen 1978).

Definitive evidence of myosin and then of actin-myosin interactions in plants had to wait until the development of motility assays in the mid-1980s and 1990s which led to the current view that coherent cytoplasmic streaming, as in the giant internodal cells of the Characeae, is accounted for by myosin bound to organelles driving their movement against anchored actin. Nevertheless, the roles of actin and myosin in the motility of organelles as individuals (i.e., not in bulk streaming flows) is far less clear, as for example, two reviews elsewhere in this issue point out (Buchnik et al. 2015; Hawes et al. 2015), and models might emerge that hearken back to those of Nina Allen and others where the filamentous network is itself active.

Much earlier than plants, *Physarum* was widely accepted to contain actin and myosin, beginning with Loewy's work in 1952 (Loewy 1952; Kamiya 1960; Hatano and Tazawa 1968; Shimmen 2007). This allowed biologists, such as Kamiya and Wolhfarth-Bottermann, to posit that the mechanisms for motion in *Physarum* were similar to the actin-myosin interactions being posited to explain muscle contraction by Huxley (Kamiya 1981; Wolhfarth-Bottermann 1979). Even though the exact nature of the interactions between actin and myosin were not known in *Physarum*, Kamiya, Wohlfarth-Botterman, and others began to consider how those interactions could be regulated.

By the 1970s, explaining the regulation of motion in *Physarum* became the problem of explaining the pulse of protoplasm. It was taken as a given that motion would be explained in terms of actin and myosin, but how they produced the pulsations of shuttle motion was not. Wohlfarth-Bottermann framed this problem as the search for an oscillator; "a 'pacemaker' or 'trigger' which governs contractions" (Wohlfarth-Bottermann 1979, p. 19). Building on the analogy again to muscle contraction, a number of biologists, including Kamiya, Hotano, and Wohlfarth-Bottermann, considered fluctuations in ATP and calcium as potential oscillators. Both showed some relationship to the rhythmicity of protoplasmic motion, but not enough for a definitive declaration by either Kamiya in 1981 or Wohlfart-Bottermann in 1979. Contemporary efforts by Kenji Matsumoto, Seiji Takagi, and Toshiyuki Nakagaki, for example, to study the biophysics of shuttle streaming, to develop mathematical models of oscillators, and to continue to investigate chemical

triggers like calcium fall into this latest tradition of explaining the pulse of protoplasm, but this time as a problem of cellular regulation (Matsumoto et al. 2008).

## CONCLUSION

During the first heyday of contractile explanations for protoplasmic streaming in the nineteenth century, biologists saw a natural analogy between the contractile fibers of muscle and the supposed contractile fibers in the cell. Just as circulating blood was driven by the heart's contractions, the protoplasm was driven by the cell's fibrous contractions. The revival of contraction in the mid-twentieth century also embraced the analogy to muscle contraction as Seifriz, Kamiya, and others took the pulse of *Physarum*. The major difference between these two eras lies in the approach that biologists in each took toward cellular fibers. Twentieth century observations of cellular fibers at sites of motive force combined with a macromolecular perspective led biologists to embrace explanations based first on the contractile properties of proteins and later, following muscle research, explanations based on protein interactions. Explaining the pulse of protoplasm thus moved from a problem of explaining the mechanism of contraction to a problem of explaining how contraction could be regulated to produce the ebb and flow of protoplasm.

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