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Review

Physical chemistry of actin: Past, present and future *

Fumio Oosawa
Aichi Institute of Technology, Toyota 470-03 (Japan)

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Abstract

History of actin research is reviewed with special emphasis on dynamics of the G–F transformation and flexibility or intrafilamentous mobility of F-actin. Good correlation was found between the flexibility of F-actin and its activity in cell motility. In molecular machines such as the flagellar motor and the sliding machine of F-actin and myosin, the coupling between influx and efflux seems to be loose. F-actin would assume multiple active states during sliding on myosin with hydrolysis of ATP. Recently, the three-dimensional structure of actin molecule in crystals has been determined. Actin research is expected to give an answer to the question on the physiological significance of internal mobility of protein molecules and their assemblies and the structural origin of such mobility.

Keywords: Actin; G–F Transformation; Muscle dynamics; Sliding machine; Loose coupling

1. Introduction

Actin was discovered by F.B. Straub fifty years ago as one of the major components of muscle proteins and as a partner of myosin for superprecipitation with ATP [1].

Now, we know actin is widely distributed in almost all kinds of living cells and involved not only in muscle contraction and other cell motilities but also in various cellular activities and their regulation. The research on actin has been greatly extended and occupies a central part of cell biology.

When I intended to begin the study on actin nearly forty years ago, I did not expect such a great extension.

The first finding on actin by Straub was its G–F transformation. Actin is in the state of globular molecules named G-actin in water and is transformed into fibrous polymers named F-actin upon addition of neutral salts. With removal of salts, actin returns to the state of G-actin.

His second finding in 1950 was that G-actin has bound ATP and this ATP is hydrolysed during the G–F transformation; ADP produced is kept bound in F-actin [2]. The reverse transformation does not bring about rephosphorylation of ADP; G-actin releases ADP and gains new ATP in solution.

Very recently, the research on actin has made a big progress. It is determination of the structure of actin molecule at the atomic resolution by X-ray crystallography in Heidelberg [3] and proposal of the structure of F-actin also at the atomic resolution based on the molecular structure and X-ray diffraction data, by K. Holmes et al. [4].

* This article is based on the author's lecture given at the Conference "ACTIN 92", August 1992 in Albany, NY.

We are now having a structural basis to understand biological functions of actin.

What did happen between the initial stage and the present stage of actin research? Here, I should like to describe my personal history of actin research and present my interests for future research.

2. Background

I began the experiment on actin in 1954. I had been working in the field of polyelectrolytes. Polyelectrolyte gels were proposed as a model of muscle by W. Kuhn and A. Katchalsky in 1950 [5]. A gel of chain molecules of polyacid was extended by addition of alkali and contracted by addition of acid. Chemical free energy produced by neutralization of acid and alkali was converted into mechanical work.

In the same period, I learnt from a book of Szent-Gyorgyi that superprecipitation, which was regarded as an *in vitro* muscle contraction, required combination of two proteins, myosin and actin, both in the state of aggregates [6]. In the case of polyelectrolyte gels, contraction of a macroscopic gel was due to contraction of individual chain molecules. However, Szent-Gyorgyi's experiments suggested that muscle contraction is not due to contraction of individual protein molecules, but probably due to a change in the structure formed by two kinds of assemblies of protein molecules.

The polyelectrolyte gel did not represent the muscle. In order to understand the mechanism of muscle contraction, we needed to study muscle fibers or muscle proteins directly.

We, five young physicists in Department of Physics of Nagoya University, decided to start from molecules of muscle proteins. Our questions were; what kind of assemblies are made by those protein molecules; how are these assemblies formed; what is the minimum requirement of the structure made by those assemblies for "contraction"; and, finally, what kind of structural changes are involved in "contraction".

In the autumn of 1954, rabbits were introduced in our laboratory. It was a big event for people in physics. At that time, we even did not have an electric refrigerator in our laboratory. Instead, we had various home-made instruments for polymer physics, instruments for measurement of light scattering, flow birefringence, viscosity, osmotic pressure, etc.

3. The G–F transformation

The method of preparation of actin from living muscle invented by Straub seemed not to be difficult for us. Preparation of myosin absolutely required a cold room. Moreover, we were most interested in the G–F transformation of actin because of our experience in polymer physics. We began preparation of actin.

Our primary aim was to make a map on the state of actin in all possible environmental conditions.

Our first paper on the G–F transformation of actin was published in 1959, five years after our start, although preliminary results had been reported at the International Conference on Muscle Biochemistry, held in Tokyo in 1957 [7]. The main findings were summarized as follows:

1. Presence of a critical concentration of G-actin is required to form F-actin.
2. Microscopic cycling of actin molecules occurs between two states, G-actin and F-actin.
3. Polymerization of G-actin consists of nucleation and growth.

In pure water, all actin molecules are in the state of G-actin and in a physiological concentration of salts, all are in the state of F-actin. What does happen in intermediate concentrations of salts? By changing the concentration of actin, we found that below a certain concentration of actin, no F-actin is formed; and above this concentration, F-actin begins to be formed. The F-actin formed coexists with G-actin, the concentration of which is kept constant at the critical concentration. (See Fig. 1.) The phenomenon observed was similar to gas–liquid condensation; F-actin corresponds to the liquid and G-actin at the critical concentration corresponds to the gas at saturated vapor pressure. The critical concentration decreases with increasing salt concentration.

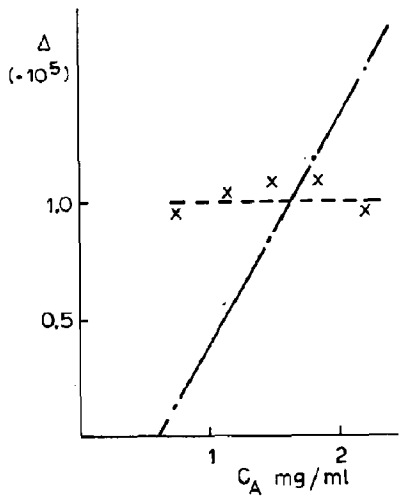

Fig. 1. A condensation-like feature of the G-F transformation of actin. The relation between the degree of flow birefringence (•) (ordinate) showing the amount of F-actin and the actin concentration (abscissa) at an intermediate concentration of salts ($MgCl_2 = 0.7$ mM), and the degree of flow birefringence of the solution after ultracentrifugation to remove F-actin and addition of salts (×), which shows the amount of G-actin coexisting with F-actin before ultracentrifugation. (From ref. [7].)

Now, everyone knows actin in solution is a mixture of G- and F-actins. However, it was an important question to be answered; whether actin in an intermediate concentration of salts has an intermediate structure between G- and F-actins or is a mixture of two extremes, G- and F-actins. Our experiments gave the answer.

Coexistence of G- and F-actins suggested that both G to F and F to G transformations occur at the same intermediate concentrations of salts. This means that each actin molecule undergoes cyclic changes between G and F. Fortunately, the G–F–G cycle was expected to result in the hydrolysis of ATP. Such a continuous hydrolysis of ATP was actually observed after establishment of the G–F balance in intermediate concentrations of salts [8]. The macroscopic balance between G- and F-actins is supported by the microscopic cycling of actin molecules.

Our finding indicated that statistical thermodynamics can be applied to the G–F transformation of actin, although irreversible ATP hydrolysis is involved.

In addition, the kinetic analysis showed that the G–F transformation consists of two steps, nucleation and growth; in ordinary conditions of the environment, the first step, nucleation, is rate-limiting. This was another condensation-like feature of G–F transformation.

4. Structure of F-actin

On the basis of the condensation-like feature of G–F transformation, we constructed a theory of helical polymerization of protein molecules and presented a helical polymer model for F-actin [9–11]. In that model, each actin molecule is bound with four neighboring molecules through two kinds of bonds, except molecules at both ends [9]. (See Fig. 2.) All of our experimental data were understood according to this model.

Shortly after our proposal, J. Hanson and J. Lowy gave electronmicrographs of negatively stained F-actin in 1963, which showed a two stranded helical polymer structure [12].

Since two kinds of bonds were assumed between actin molecules in F-actin, we imagined structural changes of F-actin caused by partial

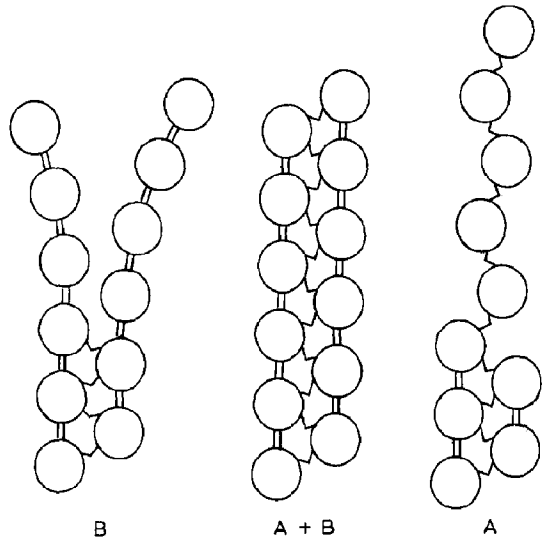

Fig. 2. A model of F-actin, showing a helical polymer formed by two kinds of bonds between neighboring molecules (middle); Partial breaking of one of the two kinds of bonds produces linear polymers (right and left). (From ref. [9].)

breaking of bonds. If one of the two kinds of bonds were broken, linear polymers would be formed. Or, weakening and strengthening of each bond would result in conformational changes of F-actin. Recent structural analysis supports the idea of two kinds of bonds in F-actin, one along the longitudinal strands and the other between two strands.

Thus, two characteristics of actin are: (1) the G–F transformation as a kind of condensation which is regulated by the environmental condition, and (2) the helical polymer structure of F-actin which has the possibility of conformational changes keeping structural continuity.

We had two questions. What is the physiological significance of the G–F balance and its regulation? What is the physiological significance of the helical polymer structure of F-actin and its conformational changes?

5. Non-muscle actin

It seemed unlikely that the G–F transformation of actin is directly involved in muscle contraction. The filamentous structure is stable in muscle fibers, particularly in the skeletal muscle. On the other hand, in many non-muscle cells, formation and destruction of filamentous structures were often observed, which had been called sol–gel transformation. Therefore, we intended to extend our research to non-muscle cells. Two cells were chosen; *Physarum plasmodium* and sea urchin egg.

Physarum plasmodium shows vigorous shuttle streaming of protoplasm. It appeared to be due to a contraction-extension cycle of gel structures composed of protein filaments. Before I started experiments on actin, I had frequently visited N. Kamiya's laboratory at Osaka University to enjoy observation of protoplasmic streaming under an optical microscope. He showed many beautiful experiments on oscillatory force in plasmodium. S. Hatano from his laboratory joined us in the new Institute of Molecular Biology of Nagoya University and began extraction and purification of actin-like protein from *Physarum*.

Dynamic behavior of protein filaments was also observable in the mitotic apparatus during cell division. T. Miki-Noumura from K. Dan's laboratory in Tokyo joined us for extraction and purification of actin-like protein from dividing cells of sea urchin egg.

Actin-like proteins in *Physarum plasmodium* and sea urchin egg cells were searched for by cross-reaction with myosin from rabbit muscle. By the end of 1961, superprecipitation was demonstrated by the complex of both actin-like proteins with rabbit myosin. However, purification of those proteins took a few years.

Actin from *Physarum* reported in 1966 was the first non-muscle actin which had full ability of the G–F transformation [13]. Pure actin was also obtained from sea urchin egg cells [14].

In muscle fibers, almost all actin molecules are in the state of F-actin. At the salt concentration *in vivo*, the critical concentration is very low. On the other hand, in non-muscle cells, G- and F-actins often coexist and their balance seems to be variable depending on the physiological state of the cell. Therefore, a question was whether such a difference in the G–F balance between muscle and non-muscle cells is due to a difference in some property of actin itself or due to a difference in other factors in the cell.

Physarum actin and sea urchin actin, when purified, were found to be very similar to muscle actin. They showed the same critical concentration as muscle actin under similar environmental conditions. Actin itself is not very different between muscle and non-muscle cells.

These two actins were very good representatives of non-muscle actin. One was from *Physarum* which shows active movements and stands very far away from higher animals in evolutionary terms. The other was from egg cells, of which the primary function is not movement. Consequently, our result suggested that actin is a highly conserved protein which is possibly distributed in many kinds of animal and plant cells with or without motility.

Thus, the difference in behavior of actin in muscle and non-muscle cells was considered to be due to some other components interacting with actin. Living cells must have a mechanism to

control the amount and distribution of F-actin depending on the physiological requirement. Later, researchers showed that many kinds of actin-binding proteins work along this mechanism. Some bind to G-actin to inhibit formation of F-actin; some break F-actin into fragments, or promote nucleation of F-actin. A big field of cell biology was opened.

6. Flexibility of F-actin

In 1954, muscle contraction was found to be due to sliding between thin filaments of F-actin and thick filaments of myosin interdigitating each other. Interesting ideas were proposed on the mechanism of sliding [15–17]. In none of these ideas, however, much attention was paid to internal mobility of thin filaments or F-actin.

As described previously, we were very interested in this internal mobility or ability of conformational change of F-actin. We undertook many experiments to examine such mobility; for example, the exchangeability of nucleotides or divalent cations tightly bound to F-actin was investigated at low concentrations of salts, under sonic vibration, under interaction with myosin, etc.

The first experimental evidence for flexibility of F-actin was obtained by quasi-elastic light scattering measurements by S. Fujime in 1970 [18]. Frequency broadening of laser light scattered from F-actin solutions contained contributions from thermal bending movements of F-actin. The analysis gave estimation of the flexural rigidity of F-actin; F-actin of the length of 2 μm undergoes bending movements of the amplitude of about 40 nm and the apparent period of about 10 ms [19]. Later, the statistical analysis of overall shape of F-actin in electronmicrographs yielded similar estimations of the flexural rigidity [20].

Muscle researchers did not want to believe such flexibility of F-actin. It was mainly because in the current theory of sliding, F-actin had always been treated as if it were a rigid rod.

Several years passed; finally thermal bending movements of single F-actin filaments were directly observed under an optical microscope. First, it was made possible in 1978 by dark-field optical microscopy using F-actin decorated with myosin fragments [21]. (Fig. 3.) Then, F-actin chemically or physically labelled with fluorescent dyes was found to be visible by fluorescence microscopy [22]. Previous results derived from quasi-elastic light scattering measurements were all confirmed.

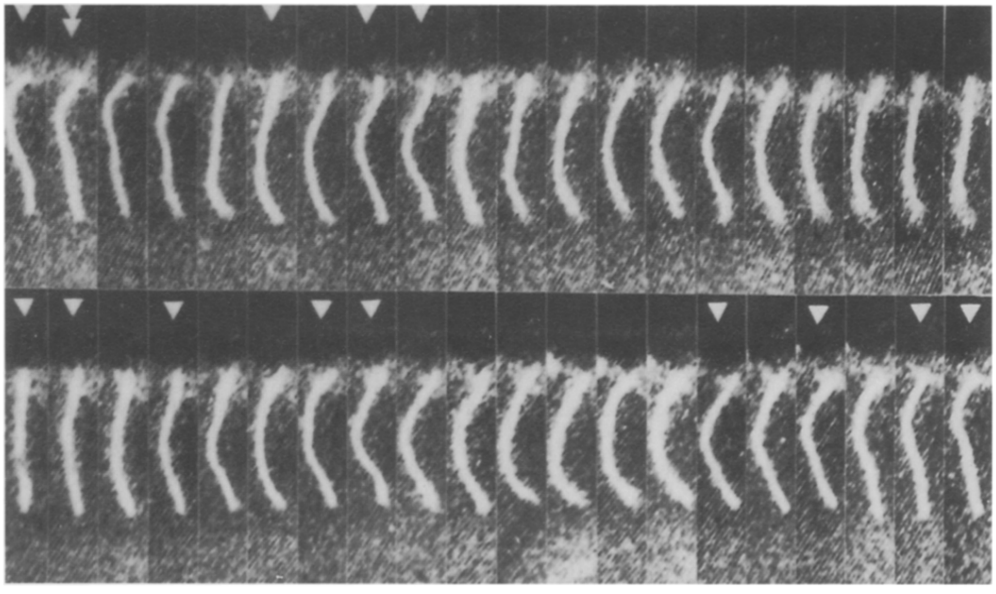

Fig. 3. Optical micrographs showing thermal bending movements of a single F-actin filament of 10 μm in length (decorated with HMM). Time interval $\frac{1}{12}$ s. (From ref. [21].)

Now, it is thought that the observed flexibility of F-actin is reasonable as a filament made by secondary bonding of globular protein molecules.

In the skeletal muscle, S. Ebashi discovered that troponin (TN) and tropomyosin (TM) are bound to F-actin to form the thin filament and interaction of F-actin in the thin filament and myosin in the thick filament for sliding is initiated by binding of Ca^{2+} to troponin [23]. Investigation of the flexibility of the thin filament or the F-actin–TN–TM complex has indicated that the complex is more rigid in the absence of Ca^{2+} than in its presence [24]. The Ca^{2+} binding to TN brings about an increase of flexibility of the complex.

Upon addition of soluble myosin fragments, HMM or S-1, with ATP, the F-actin–TN–TM complex in solution showed bending movements of large amplitude and high frequency in the presence of Ca^{2+}. Bending movements were activated by hydrolysis of ATP on myosin fragments. Such activation was not observed in the absence of Ca^{2+}.

Thus, good correlation was found between the flexibility increase and activation of the F-actin–TN–TM complex. Flexibility of F-actin or the thin filament seemed to be necessary for muscle contraction. *Why*? (This was a serious question to me.) If one imagines myosin molecules walking on F-actin during sliding, F-actin should be rigid. "For walking, solid ground would be better." (Fig. 4.)

Thermal fluctuation in twisting of two strands in F-actin was confirmed by electronmicrographs and phosphorescence anisotropy measurements [25,26]. Recently, the rigidity against stretching or extensibility of F-actin was also directly measured in solution. The extensibility obtained appeared to be unfavorable for the current model of sliding where elasticity was assumed only in the myosin molecule.

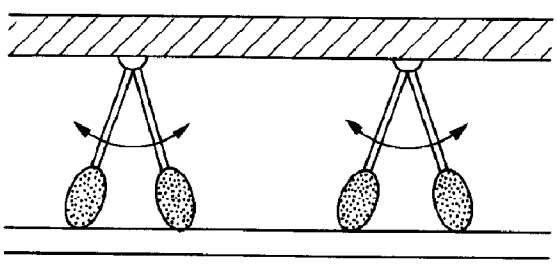

Fig. 4. Walking molecules on a rigid rod (solid ground).

7. The loose coupling

The sliding machine of F-actin and myosin is a microscopic one. The following problem came to my mind; what kind of mechanism is working in the machine, if the free energy to be converted is of the same order of magnitude as the energy of thermal fluctuation. A positive role of internal mobility of F-actin must be hidden in here somehow.

Since 1964, my colleague S. Asakura was developing research on bacterial flagella, the other kind of protein polymers [27]. Then, our interests were extended to motility of bacterial cells. They swim in water by a rotating flagellum of helical shape (micropropeller).

About twenty years ago, a rotary motor-like structure was discovered at the base of each flagellum. In 1977, it was found in Nagoya, and also in the United States, that this motor is driven by a flux of protons from outside to inside the cell according to the electrochemical potential gradient [28–30]. Bacterial cells usually have a negative inside electric potential of about -0.2 V. The speed of rotation of the motor is about 100 Hz; in some cells it attains 800 Hz [31]. This corresponds to a linear speed of about 10 μm/s at the "rotor". The force to drive the "rotor" is of the order of 10 pN. These values of speed and force are of the same order as those in the sliding between thin and thick filaments in muscle.

An electrochemical potential gradient of protons was artificially applied to a cell after starvation. The motor showed the following characteristics. The proton energy given by the electric potential difference across the membrane and the negative entropy ($\times T$) of protons given by the pH difference across the membrane are both equivalent to drive the motor as two components of free energy. With decreasing electric potential difference or pH difference, the motor decreases

the speed of rotation, continuing smooth rotation [30].

At the electric potential difference of 25 mV, where the free energy of proton utilizable for rotation is equal to the average energy of thermal fluctuation at room temperature, kT, the motor rotates slowly and steadily. Even when the ratio of concentrations of proton outside to inside is 3:1, which is equivalent to an electric potential difference of 30 mV, rotation of the motor does not show much fluctuation.

Such behaviors of the motor suggest that flow of individual protons in the motor is not tightly coupled with rotation of the motor. It is unlikely that the motor is simply a rigid mechanical machine. It must have internal mobility to perform some statistical procedure for smooth conversion of small free energy in large thermal fluctuation.

During the 1980s, I proposed "loose coupling" between proton flux and motor rotation [32,33]. Most of the models on the motor mechanism proposed by other researchers assumed tight coupling. The idea of loose coupling was not easily accepted. However, now our field has come to understand the significance of this question, i.e. whether the coupling is tight or loose.

Returning to the problem of the sliding machine of F-actin and myosin, I thought, the flexibility or internal mobility of F-actin must be important because free energy to be converted is not much larger than the energy of thermal fluctuation.

8. The sliding machine and its energetics

The coupling between hydrolysis of ATP and sliding was first investigated by T. Yanagida et al. using a glycerinated fiber of crab leg muscle [34]. Each thin filament ws made free to slide between thick filaments by removing z-lines by enzymatic digestion. After addition of ATP, the sliding speed of thin filaments fluorescently labelled was measured under an optical microscope. In the same setup, the hydrolysis of ATP was followed.

The sliding distance of the thin filament produced by hydrolysis of one ATP molecule was estimated to be about 60 nm. Under isometric conditions, sliding does not occur, although ATP is hydrolysed. Therefore, the sliding distance per ATP molecule is variable from 0 to 60 nm with decreasing load. The distance of 60 nm is very much larger than the size of myosin head or actin molecule. This result, reported in 1985, was far beyond our imagination. The coupling between hydrolysis of ATP and sliding is extremely loose.

Then, the system was simplified. As described before, F-actin interacting with myosin fragments, HMM or S-1, in solution does not show sliding movements upon addition of ATP. They show only active bending movements [22]. However, when HMM or S-1 was fixed on a glass plate, F-actin interacting with them showed unidirectional sliding movements [35]. That is, single F-actin filaments and myosin heads fixed on a plate compose the simplest sliding machine.

Such sliding movements had been observed in the case of F-actin bundles extracted from a cell of *Nitella*, which exhibits fast circulation of cytoplasm, by S. Higashi-Fujime in 1980. The speed exceeded 30 μm/s [36]. It was very likely that the bundle was moved by interaction with myosin-like molecules of *Nitella* attached to a glass slide by chance. One myosin molecule, if existant on the slide, would be enough to produce a force to drive the bundle against viscous friction. However, we had to notice that if the number of myosin molecules was small and the sliding distance per ATP hydrolysis was of the same order as the size of myosin head, the observed high speed could not be realized.

The sliding distance per ATP hydrolysis has been determined in the system of single F-actin filaments and myosin heads fixed on a plate. The distance has been found to be even longer than 60 nm [37]. Again, extremely loose coupling. Such loose coupling suggests the possibility that free energy of ATP hydrolysis may be divided into small pieces that are gradually released during sliding. The free energy used for sliding of the distance of one actin molecule (5 nm) would be of the same order as, or be smaller than, the average energy of thermal fluctuation (1 kT).

9. A flexible ratchet

Even when F-actin is so short that it can interact simultaneously with only a few myosin heads, it shows continuous sliding at a high speed. The inertia of F-actin moving in water is negligibly small as compared with viscous friction.

Let us suppose skating on ice. Usually, inertia is fully utilized for skating at a constant speed. Without inertia, how is a constant speed maintained? One may imagine small vibrational movements at the interface between shoes and ice, and directional friction between them. It is a ratchet mechanism.

A microscopic ratchet mechanism was discussed in Richard Feynman's Lectures in Physics [38]. There, two assumptions were put for unidirectional rotation of a ratchet; a temperature difference between a ratchet and a pawl, and a structural asymmetry of the ratchet. Microscopic thermal movements, rotational in the ratchet and longitudinal in the pawl, were considered. The temperature difference produces a flow of energy, if the ratchet is at a higher temperature, from the ratchet to the pawl. In the case of Feynman's ratchet, which was rather rigid, the amount of energy consumed as heat per unit rotation was independent of the speed of rotation or the load.

We may assume some flexibility in the ratchet [39]. For example, the height of the teeth of the ratchet may be variable. (See Fig. 5.) If at high speed of rotation the average height is low and at low speed it is high, the energy consumed as heat becomes dependent on the speed or load, as expected in the case of loose coupling.

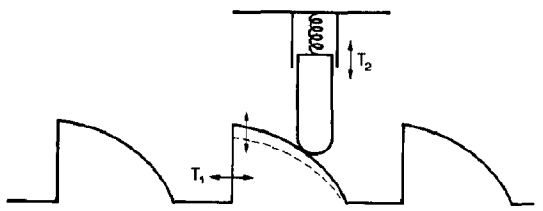

Fig. 5. A flexible ratchet as a possible model of the sliding mechanism.

Such a flexible ratchet can give a load–velocity relation and a heat rate–velocity relation both similar to those observed in muscle fibers.

I should like to imagine that F-actin would correspond to the ratchet and myosin head to the pawl, although the reverse would be possible too. "High temperature" may mean that high energy coming from hydrolysis of ATP on myosin head is stored and distributed in several degrees of freedom in the structure of F-actin, being exchangeable among them.

10. Multiple states of F-actin

Briefly summarizing, the sliding machine of F-actin and myosin has two remarkable characteristics: (1) loose coupling between hydrolysis of ATP and sliding, and (2) continuous sliding at a high speed under zero load. As discussed above, these characters suggest that F-actin and myosin head possess internal mobilities and assume many states of different free energies. My interests lie in the question of how many states F-actin can assume.

It had been long believed that the hydrolysis of ATP bound to G-actin is obligatorily coupled with the G–F transformation. Later, however, G-actin with ADP or even without nucleotides was shown to form F-actin [40,41]. M. Carlier and E. Korn found that when G-actin molecules having ATP are incorporated into F-actin, they keep ATP for a while and then hydrolyse it into ADP and inorganic phosphate to finally release inorganic phosphate [42,43]. There are three kinds of actin molecules in F-actin, those having ATP or ADP and inorganic phosphate (P_i), or ADP alone; near the ends of F-actin more molecules having ATP. They have different binding free energies in F-actin, so that they must be in different conformations. In F-actin having ADP and P_i, loosening of bonds between two strands has been noticed in electronmicrographs [44].

F-actin has polarity. Two ends, named the barbed end and the pointed end, respectively, have different kinetic constants of association and dissociation of actin molecules. In a stationary state, polymerization at the barbed end and de-

polymerization at the pointed end take place simultaneously. This phenomenon called "treadmilling" is possible because of involvement of hydrolysis of ATP [45]. However, the difference of kinetic constants at two ends was observed even when hydrolysis of ATP was not involved. Conformations of actin molecules at or near one of the two ends are not the same as those at or near the other end.

Kouyama and Mihashi found that the fluorescence of pyrene derivatives bound to actin molecules increases very much with the G–F transformation [46]. This fluorescence increase has been a best indicator of transformation. According to direct observation of single F-actin filaments, the fluorescence intensity of derivatives labelled to them has a small gradient along the filament. The influence of the ends seems to be extended toward the middle of the filament. I would like to suppose that the physical state of actin molecules in F-actin is not uniquely determined by the chemical state of bound nucleotides.

Many experiments have been undertaken to detect changes in the state of actin molecules in F-actin interacting with myosin heads during hydrolysis of ATP. Some have given positive results. Observation of active bending movements of F-actin described before is one of them. The amplitude and frequency of bending movements were apparently equivalent to those expected at a hypothetical temperature three or four times higher than room temperature. Free energy obtained by hydrolysis of ATP on myosin heads was transferred, at least partially, to degrees of freedom of bending movements of F-actin. The energy was not instantaneously consumed as heat. It would be usable for sliding, if F-actin were interacting with myosin heads fixed on a plate.

Anyway, in order to explain the above-mentioned characters of the sliding machine, we need to assume multiple active states, instead of a single high energy state, in F-actin and myosin head. From this standpoint, we have to analyse what kind of changes is induced in actin molecules in F-actin by hydrolysis of ATP on myosin head, and estimate the life time and spatial range of such changes.

11. Cytoskeletal regulation

Actin has been found in almost all kinds of cells. Regulation of the G–F balance and the length, polarity, and localization of F-actin is a key process in cellular morphology and movements. Besides the sliding, I like to notice non-sliding motility of actin in the cell.

In some cells, the G–F transformation of actin directly gives a force to move intracellular structures [47]. Fragmentation and cross-linking of F-actin often change cellular morphology. Conformational changes of F-actin due to partial breaking–formation or weakening–strengthening of bonds may also be involved in intracellular movements. Many actin-binding proteins participate in these processes. Interaction of these proteins with actin is spatially and temporarily coordinated in response to external and internal signals. Chemical modification of actin molecules may be one of the ways of regulation of the amount and mobility of F-actin [48,49].

These problems, however, are outside the scope of this article.

12. Other protein polymers and molecular machines

Historically, actin research gave a theoretical framework to understand the polymerization of other proteins. The polymerization of bacterial flagellin molecules to flagella has similar features as the G–F transformation of actin [27]. There is a critical concentration of flagellin molecules to form flagella *in vitro*. The polymerization consists of nucleation and growth; in ordinary conditions, nucleation does not occur spontaneously. The finding that growth of flagella is exclusively unidirectional promoted investigation of directionality of growth of F-actin.

The polymerization of tubulin molecules to microtubules also has similar features, although the process is a little more complicated—GTP and GDP take the same role as ATP and ADP in actin. Delay of hydrolysis of GTP was observed after tubulin molecules having GTP are incorporated into microtubules. If tubulin molecules in

the ends of microtubules have GTP, their rate of dissociation is very slow; if they have GDP, it is fast. Under a certain environmental condition, long-range polymerization and depolymerization take place alternatively in single microtubules [50,51]. This phenomenon, named "dynamic instability", was observed also in F-actin in a less pronounced manner.

The kinetic theory which was presented to describe the nucleation-growth process of F-actin has been successfully applied even to the process of formation of three-dimensional crystals of protein molecules [52,53]. The theory has been useful, for example, to estimate the size of nuclei of those crystals.

We have only one case of protein polymers, in which bonds or interaction between neighboring molecules were clarified in terms of atomic coordinates. It is TMV (tobacco mosaic virus) [54]. In this case, we know the conformational change of unit protein molecules required to form the polymer. The structural analysis of bacterial flagella at atomic resolution is now in progress [55]. Correlation between the conformational change of flagellin molecules required and the directionality of growth of flagella has been suggested in an experiment using partially digested flagellin molecules. Similar correlation is expected in the G–F transformation of actin.

F-actin is a flexible filament. The flexibility of bacterial flagella and microtubules has been also measured.

They have many longitudinal strands to form tubular polymers. Therefore, they are much more rigid than F-actin. However, their rigidity does not necessarily mean that they have no internal mobility.

Microtubules and dynein or kinesin compose a sliding machine similar to that of F-actin and myosin. In the case of kinesin, a simplest machine was constructed; that is, a single microtubule and a single kinesin molecule fixed on a plate [56]. The direction of sliding of microtubules on kinesin and that on dynein are opposite to each other. Kinesin and dynein interacting with microtubules have different kinetics of hydrolysis of ATP [57].

We can ask the same question about these sliding machines; i.e. whether the coupling between ATP hydrolysis and sliding is tight or loose. It is very likely that the answer is "loose", just as for the sliding machine of F-actin and myosin. I expect that there is a hidden general principle in the sliding mechanism in all of these machines, although in details the mechanism may be different in different machines.

The same question on the input–output or influx–efflux coupling can be raised in all kinds of molecules machines in living cells. Let us consider ion pumps, for example, a proton pump. Influx is the proton current and efflux is the hydrolysis or synthesis of ATP. Usually, it is thought that the ratio of the number of transported protons to the number of hydrolysed or synthesized ATP molecules is (or should be) an integer. Actually, the ratio reported was mostly three, even if the experiment did not exactly give an integer. I would like to suppose the possibility that the ratio be really non-integer [33]. If a loose coupling mechanism were working in the machine, the ratio could be non-integer and variable depending on the experimental condition. Intramachine mobility may make the coupling loose. We need more experiments.

How about more chemical machines; the machine for synthesis of polypeptide along mRNA on ribosome, or the machine for synthesis of RNA along DNA. Sliding-like movements are involved in these machines. The influx–efflux coupling must be reexamined.

13. Concluding remarks

Actin has represented a group of many proteins which have similar functions. Actin research has given much influences to research of these proteins and their systems. Understanding of morphogenesis and motility of various intracellular structures has made a big progress.

Three years ago, the atomic coordinates of actin molecules in crystals of its complex with actin-binding proteins have been determined. Based on these coordinates, a model of F-actin at atomic resolution has been proposed to get a best

fit to the X-ray diffraction pattern from F-actin gel, although the structure of an actin molecule in F-actin must be more or less different from that in crystals. In addition, mutants of actin molecule can be produced by modification of DNA.

In this sense, we are now standing at a new starting point of actin research. We have to try to find the structural basis of all observed properties of actin. In the case of actin, dynamics and energetics in the structure must be particularly important for its functions. Therefore, it will not be easy to investigate the structure–function relationship in actin. Nevertheless, I expect that actin research will give an answer to the question of how internal mobility of protein molecules and their assemblies is useful in their functioning.

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