

# Unidirectional Sliding of Myosin Filaments along the Bundle of F-Actin Filaments Spontaneously Formed during Superprecipitation

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**ABSTRACT** I reported previously (Higashi-Fujime, S., 1982, *Cold Spring Harbor Symp. Quant. Biol.*, 46:69–75) that active movements of fibrils composed of F-actin and myosin filaments occurred after superprecipitation in the presence of ATP at low ionic strengths. When the concentration of  $MgCl_2$  in the medium used in the above experiment was raised to 20–26 mM, bundles of F-actin filaments, in addition to large precipitates, were formed spontaneously both during and after superprecipitation.

Along these bundles, many myosin filaments were observed to slide unidirectionally and successively through the bundle, from one end to the other. The sliding of myosin filaments continued for  $\sim 1$  h at room temperature at a mean rate of  $6.0 \mu m/s$ , as long as ATP remained in the medium. By electron microscopy, it was found that most F-actin filaments decorated with heavy meromyosin pointed to the same direction in the bundle.

Myosin filaments moved actively not only along the F-actin bundle but also in the medium. Such movement probably occurred along F-actin filaments that did not form the bundle but were dispersed in the medium, although dispersed F-actin filaments were not visible under the microscope. In this case, myosin filament could have moved in a reverse direction, changing from one F-actin filament to the other. These results suggested that the direction of movement of myosin filament, which has a bipolar structure and the potentiality to move in both directions, was determined by the polarity of F-actin filament in action.

Various types of cell motility are based on actin and myosin. A specific structure of motile systems composed of actin and myosin is organized in a particular period and at a particular locus of the cell depending on the cellular activity. Each different type of motility must be presented by a different structure of the actomyosin motile system. Many kinds of actin-binding proteins found so far (for a review, see reference 20) are thought to organize and regulate the structure of the motile system. Phosphorylation of myosin heavy chain (5, 25) and light chain in nonmuscle cells (1, 19, 39) may be another regulatory mechanism.

The cytoplasmic streaming in *Nitella* cells is an example of actomyosin-based cell motility. Many cellular operations have been attempted: centrifugation of a whole cell (4), perfusion of the vacuole (34), local irradiation of the cell with ultraviolet light (2, 3), and so on. Such operations have given results suggesting that the interaction of F-actin cables located in the ectoplasm with myosin located in the endoplasm produces

the force for streaming. In fact, the rotatory movement, which is associated with F-actin bundles, of a chloroplast squeezed out of the cell was reactivated by the addition of muscle heavy meromyosin (HMM)<sup>1</sup> and ATP (21). Recently, we found that isolated chains of chloroplasts linked by F-actin bundles moved in an artificial medium containing ATP (11). Furthermore, isolated chloroplast-free fibrils and rings consisting of F-actin filaments continued to travel and rotate, even after extensive washing with the well-defined fresh medium (11). In the living cell, it is postulated that the endoplasm containing myosin is flowed by interacting with stationary F-actin bundles located at the interface between the endoplasm and the ectoplasm. After isolation, F-actin bundles can move freely. Recently, another motile system was constructed by the combination of F-actin cables from *Nitella* cell and myosin-coated polystyrene beads (27, 28). The beads moved

<sup>1</sup> Abbreviation used in this paper: HMM, heavy meromyosin.

along F-actin cables in the presence of ATP.

These *in vitro* systems are very useful. However, they still contain unknown factors derived from the cell. If unknown components interfere with movement, purified proteins must be used for the *in vitro* reconstitution of the motile system.

Very recently, Yanagida et al. (36) observed the movement of single F-actin filaments in the presence of HMM and ATP. They found that F-actin filaments showed larger and faster bending movements after addition of HMM than before, but they failed to observe sliding-like movements.

We previously reported that bundles composed of F-actin and myosin filaments showed active sliding-like movements in the presence of ATP at low ionic strengths (12). The bundles repeated formation and dispersion as long as ATP remained in the medium. This *in vitro* movement closely resembled the movement of the cytoplasm in an endoplasmic droplet squeezed out of the *Nitella* cell.

By slight modification of the medium used in the experiment mentioned above, the unidirectional movement of short myosin filaments along the bundles composed of F-actin filaments was effected. This movement is the subject of this paper. After superprecipitation in the presence of MgCl<sub>2</sub> at a high concentration, F-actin bundles were formed spontaneously, and myosin filaments were observed to move along the F-actin bundles. The bundle was confirmed to have a polar structure by electron microscopy. This structural polarity of F-actin bundles explained the unidirectionality of movement of myosin filaments along the F-actin bundle. Another significant result was that myosin filaments also moved along dispersed, single F-actin filaments that were not visible by dark field microscopy. Myosin filaments could move in reverse, probably by changing the tract from one F-actin filament to another. This system is actually an *in vitro* system reconstituted by pure actin and myosin from muscle to simulate the movement in nonmuscle cells, in particular, the cytoplasmic streaming in *Nitella* cells.

## MATERIALS AND METHODS

**Preparation of Muscle Proteins:** Actin was prepared from rabbit skeletal muscle by the method of Ebashi (6) with slight modification (13). Sometimes actin was treated with 0.6 M KCl according to Spudich and Watt (29). This treatment did not make any difference in the results. Purified G-actin was polymerized at 1 mM MgCl<sub>2</sub> to F-actin before use. Myosin was prepared from rabbit skeletal muscle according to the ordinary method of repeated precipitation at low ionic strength and solubilization at high ionic strength (26). After the myosin solution was centrifuged at 23,000 rpm for 60 min at an ionic strength of 0.3 M, the supernatant was diluted to an ionic strength of 0.04 M, and the precipitates at low speed centrifugation were dissolved in 0.47 M KCl containing 60 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> buffer, pH 6.7 (this solution was denoted as K-phosphate buffer) and clarified by centrifugation at 100,000 g for 3 h. Myosin was used within a week after preparation or stored in 50% glycerol that contained 2 mM EDTA and 0.5 mM dithiothreitol (DTT) for several months. Tropomyosin was prepared according to the method described previously (13). HMM was prepared according to Szent-Györgyi (32).

**Composition of the Medium for the Unidirectional Movement of Myosin Filaments:** The formation of bundles of F-actin filaments and the unidirectional movement of myosin filaments along these bundles took place when a high concentration of MgCl<sub>2</sub> was added to the medium previously used to observe the active movement of fibrils composed of F-actin and myosin filaments (12). We usually used the following conditions: F-actin was dissolved in a solution (buffer A) containing 1.5 mM ATP, 1 mM EGTA, 4 mM MgCl<sub>2</sub>, 0.025 mM CaCl<sub>2</sub>, 30 mM Tris-HCl of pH 7.6, the concentration of MgCl<sub>2</sub> was raised to about 20 mM, then myosin was added to make a final concentration of 0.4 mg/ml myosin, 0.2 mg/ml F-actin, and about 10 mM KCl. Movement was observed at room temperature (18°–22°C). Upon addition of myosin, superprecipitation started and the movement of

myosin filaments along the bundle of F-actin filaments was observed, as described later.

The concentrations of KCl and MgCl<sub>2</sub> could be varied in the range from 5 to 15 mM and from 20 to 26 mM, respectively. However, a particular combination of concentrations of MgCl<sub>2</sub> and KCl was required. The concentration of MgCl<sub>2</sub> had to be increased with increasing concentrations of KCl. At a particular MgCl<sub>2</sub> concentration when the bundles were formed, an increase in KCl concentration resulted in a decrease in the number of the bundles and an increase in the number of dispersed myosin filaments that did not precipitate into aggregates during superprecipitation. The number of F-actin bundles increased with increasing concentrations of F-actin.

If myosin was stored in K-phosphate buffer in an ice bath, its activity as related to movement was retained at least for 1 wk. However, if myosin was stored in 0.5 M KCl instead of K-phosphate buffer, in a few days after preparation we often failed to observe the sliding of myosin filaments or the movement of actomyosin fibrils reported previously (12).

**Image Recording:** Dark field microscopy was performed by the method described previously (11). Since the intensity of light scattered from a short myosin filament was so weak, the movement could not be recorded directly on 16-mm film. Therefore, a dark field microscope was attached to a video system equipped with an ultrasensitive silicon intensifier target video camera (Ikegami SIT camera, CTC 9,000, Ikegami Tsushinki Co., Tokyo, Japan), a video tape recorder (Sony video cassette recorder J9, Sony Co., Tokyo, Japan), and a monitor (Ikegami ITC). Images were first recorded on a video tape (Sony dynamicron L-250) and then transferred onto 16-mm film (Kodak 4X, Eastman Kodak Co., Rochester, NY).

**Electron Microscopy:** Electron micrographs were taken by a JEM 100-C (JEOL Ltd., Tokyo) operating at an accelerating voltage of 80 kV. One drop of actomyosin solution in the presence of ATP was put on a carbon-coated collodion film and stained with 1% uranyl acetate. To decorate the bundles of F-actin filaments with HMM, the actomyosin solution was left at 4°C overnight to complete the hydrolysis of ATP in the medium, and then the bundles were decorated on a grid with HMM (0.1 mg/ml) dissolved in the same medium without ATP, then stained with 1% uranyl acetate.

**Measurement of ATPase Activity:** Phosphate liberated during movement of myosin filament was followed with time at 21°C, as the movement was monitored with a dark field microscope.

An aliquot of 0.2 ml actomyosin solution was added to 0.8 ml trichloroacetic acid solution (6% of the final concentration) to stop the chemical reaction at an appropriate time. After centrifugation at 3,500 rpm for 15 min, the amount of phosphate in the supernatant was determined by the method of Murphy and Riley (10, 23).

## RESULTS

### *Unidirectional Sliding of Myosin Filaments along the F-Actin Bundle*

When F-actin was dissolved in buffer A containing MgCl<sub>2</sub> at a high concentration as described in Materials and Methods, the solution did not show appreciable turbidity, but straight and thin Mg-paracrystals of F-actin were visible under a dark field microscope. The critical concentration of MgCl<sub>2</sub> for Mg-paracrystal formation was around 20 mM under the condition of pH and the composition of salts used here. Under the same environmental condition, myosin polymerized into short filaments that were observed as elliptic spots with the dark field microscope.

Just after mixing myosin with F-actin in the presence of ATP, Mg-paracrystals of F-actin were dispersed and superprecipitation started. During superprecipitation, bundles of F-actin filaments were spontaneously formed, in addition to large precipitates of actin and myosin. There still remained many myosin filaments floating in the medium. When the microscope was focused on one of the F-actin bundles, short myosin filaments were found to run out of the end of the bundle into the medium (Figs. 1 and 2). At the opposite end of the bundle, myosin filaments floating in the medium reached the bundle and then slid along the bundle (Figs. 3 and 4). Many myosin filaments moved on the bundle succes-

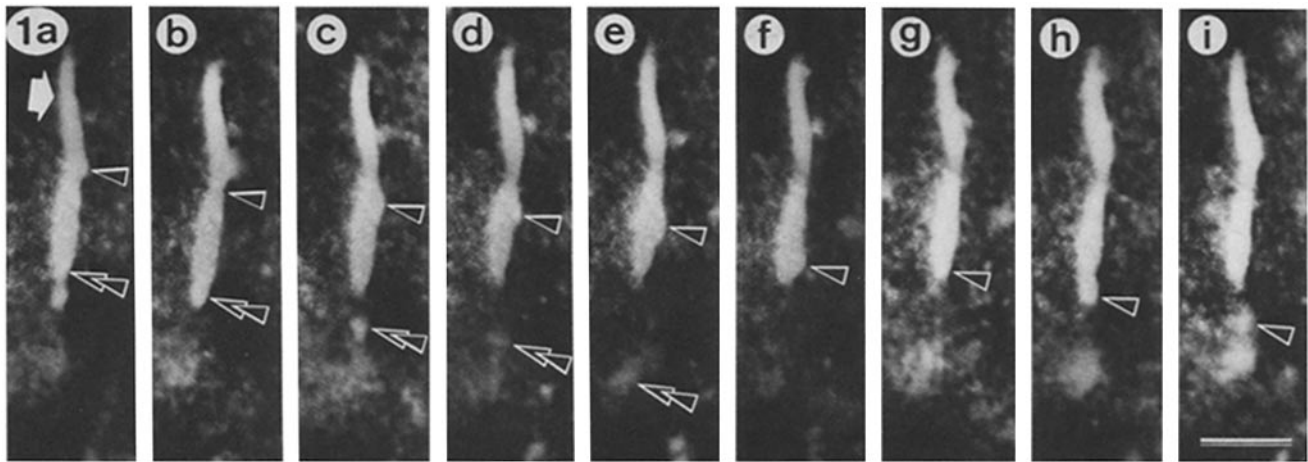


FIGURE 1 Unidirectional sliding of myosin filaments along the F-actin bundle. Myosin filaments (indicated by single and double arrowheads) were leaving the bundle after sliding along the bundle (indicated by an arrow in a). The filaments moved at the same speed both during sliding and after leaving the bundle. The medium condition was as follows: 20 mM MgCl<sub>2</sub>, 1.5 mM ATP, 1 mM EGTA, 0.025 mM CaCl<sub>2</sub>, 30 mM Tris-HCl (pH 7.6), 5 mM KCl, 0.12 mg/ml F-actin, and 0.2 mg/ml myosin at room temperature. The time interval between successive photographs was ¼ s. Bar, 5 μm. × 2,600.

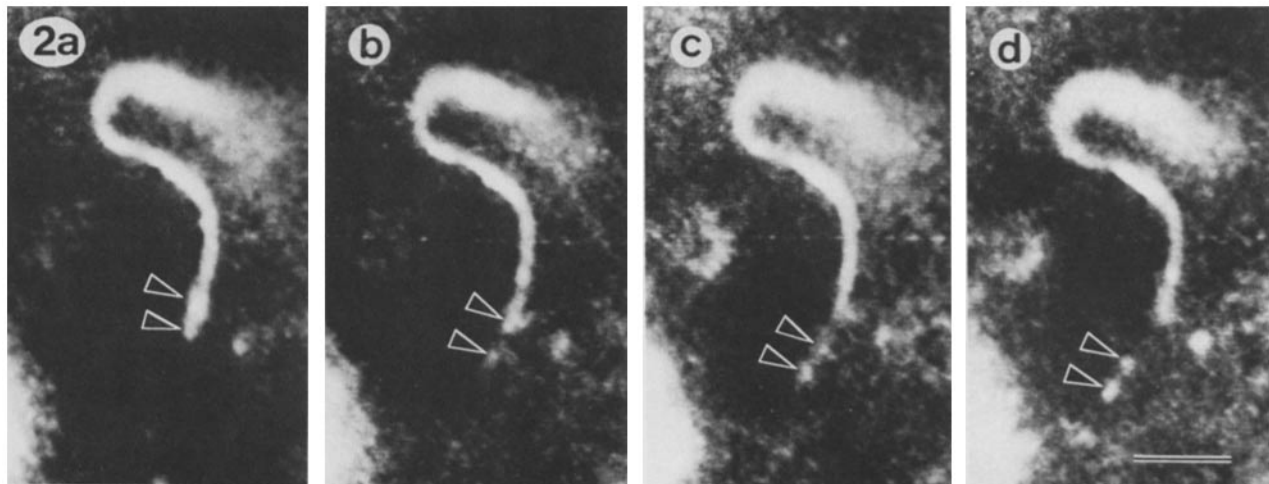


FIGURE 2 Another example of sliding of myosin filaments. Two successive myosin filaments (indicated by arrowheads) were leaving the bundle after sliding on it. The medium contained 10 mM KCl, 0.2 mg/ml F-actin, 0.45 mg/ml myosin, and the others were the same as for Fig. 1. The time interval between successive photographs was ⅙ s. Bar, 5 μm. × 2,600.

sively in a definite direction from one end to the other, and the direction of this movement was never reversed.

Directional movements of myosin filaments usually began in the medium at a certain distance from the end of the F-actin bundle (Figs. 3 and 4) and continued to a certain distance after leaving the other end (Figs. 1 and 2). This behavior suggested that the bundle was loosened at both its ends and was spread out into single F-actin filaments, and myosin filaments could slide not only on the bundle but also on single F-actin filaments although single F-actin filaments were invisible by dark field microscopy. Occasionally, myosin filaments came onto the bundle at its middle and slid on it (Fig. 5), which suggested that the middle of the bundle had a branch of F-actin filament(s).

Usually the bundle was initially straight, but it often made a large bend when a myosin filament, trapped on the way of sliding, stopped sliding temporarily. Soon after, the trapped myosin filament was released and started moving again along the bundle. After release of myosin filaments, some of the

bundles restored the straight shapes but some others kept their shapes bent as shown in Figs. 2 and 4.

The average rate of sliding of myosin filaments was 6.0 μm/s at room temperature as shown in Fig. 6a. The sliding movement continued for 30–60 min. The rate slowed down just before ATP was depleted. When ATP was depleted, no movement was observed (Fig. 7). Many myosin filaments that had been floating in the medium disappeared. They seemed to be incorporated into large precipitates or the bundles.

#### High Concentrations of MgCl<sub>2</sub>

At higher concentrations of MgCl<sub>2</sub> (28 mM for example), all Mg-paracrystals of F-actin were not dispersed during superprecipitation and some of them remained in the medium. There were no myosin filaments sliding along such Mg-paracrystals. When concentrations of MgCl<sub>2</sub> and F-actin were high (26 mM MgCl<sub>2</sub>, 0.6 mg/ml F-actin, 0.14 mg/ml myosin, and 23 mM KCl), meshworks of bundles were formed in the

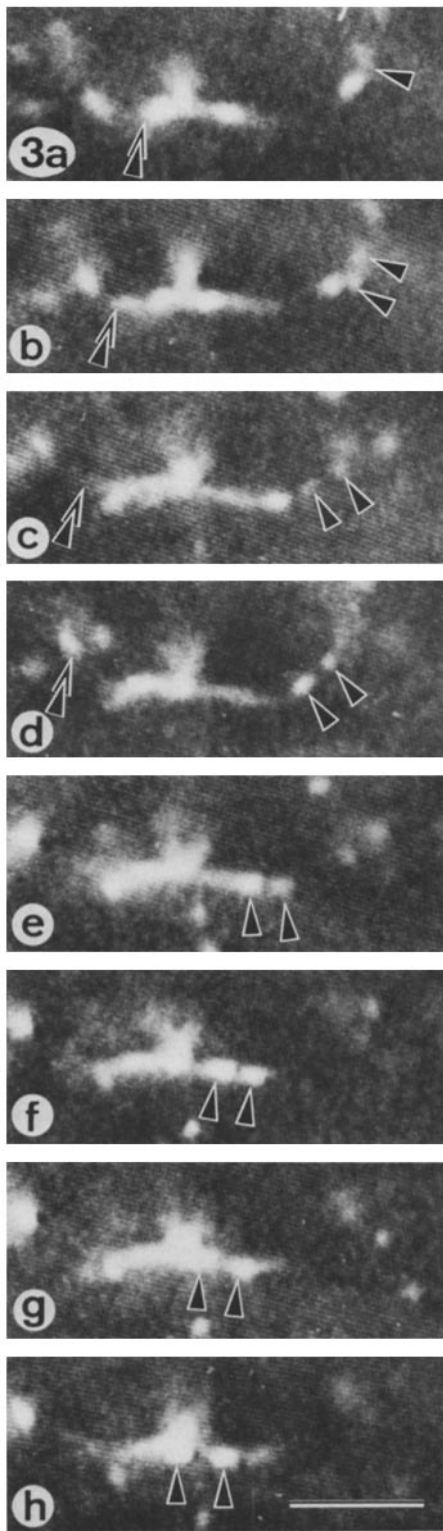


FIGURE 3 Myosin filaments coming onto the bundle. Two myosin filaments (arrowheads) came onto an end of the bundle and slid along the bundle. Another myosin filament (a double arrowhead) came out of the bundle at the other end. The condition of the medium was the same as for Fig. 2. The successive photographs were taken at a time interval of  $\frac{1}{4}$  s. Bar,  $5 \mu\text{m}$ .  $\times 3,600$ .

vicinity of the glass surface. Sliding of myosin filaments was not observed along those bundles; however, some bundles showed twitching or bending and occasionally the bundle in

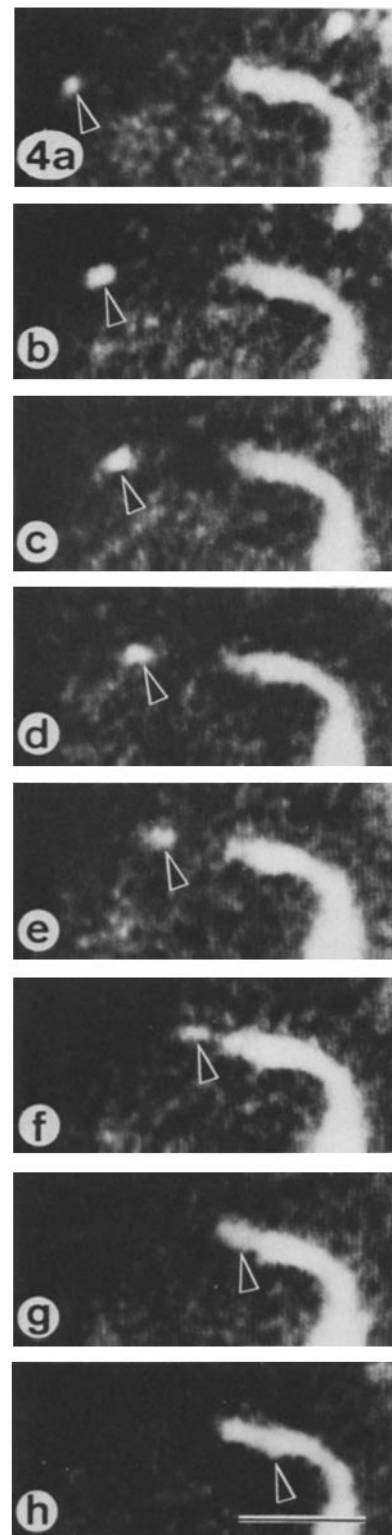


FIGURE 4 Myosin filament coming onto the bundle. A myosin filament (arrowhead) was coming onto the bundle far from the tip of the bundle, indicating the existence of F-actin filaments that might be spread out from the bundle. The myosin filament moved at constant speed before and after coming onto the bundle. The medium conditions was the same as for Fig. 2. The time interval between successive photographs was  $\frac{1}{8}$  s. Bar,  $5 \mu\text{m}$ .  $\times 3,300$ .

the meshwork generated tension to move and drag neighboring bundles by several micrometers, and then slacked immediately.

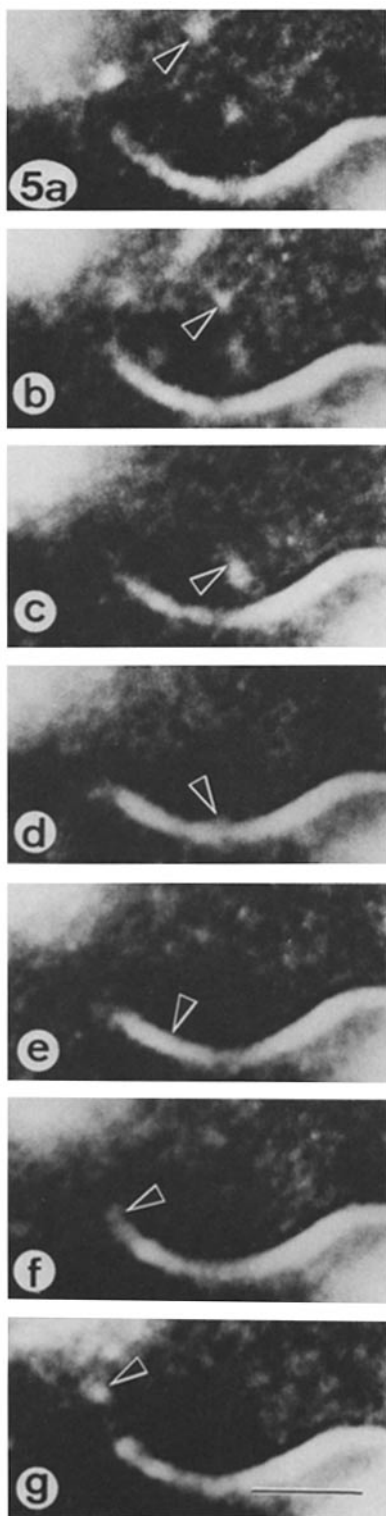


FIGURE 5 A myosin filament coming onto the bundle in the middle of the bundle. A myosin filament (arrowhead) came onto the bundle in the middle, slid along, then left the bundle. The medium condition was the same as for Fig. 2. The successive photographs were taken at a time interval of  $\frac{1}{8}$  s. Bar,  $5 \mu\text{m}$ .  $\times 2,900$ .

#### Movement of Myosin Filaments along Dispersed F-Actin Filaments

As described in the previous section, directional movement of myosin filaments before reaching and after leaving the

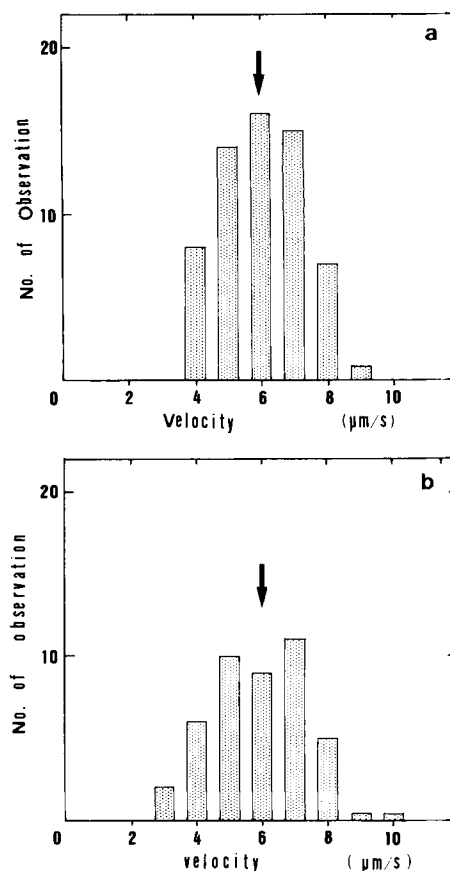


FIGURE 6 Velocity distribution of myosin movement. (a) Unidirectional sliding of myosin filaments along F-actin bundles. (b) Myosin movement along dispersed F-actin filaments. The movements were analyzed from frames of 16-mm film projected on a screen. The rate of movement of a filament varied from moment to moment, if measured accurately. The velocity was calculated from the advancement of a particular myosin filament between every two frames ( $\sim \frac{1}{8}$  s for a and  $\sim \frac{1}{4}$  s for b). We chose myosin filaments whose features could be followed clearly in frames for more than 1 s. The arrows point to the means. (a) 62 measured, mean  $6.0 \mu\text{m/s}$ , SD,  $1.0 \mu\text{m/s}$ . (b) 45 measured, mean  $6.0 \mu\text{m/s}$ , SD,  $0.75 \mu\text{m/s}$ .

bundle could be explained by sliding along single F-actin filaments spread out at both ends of the bundle. Thus, we may expect myosin filaments to slide along individual F-actin filaments dispersed in the medium.

In fact, many myosin filaments moved in the medium very quickly and straight for a long distance ( $\geq 10 \mu\text{m}$ ) as shown in Fig. 8 (left). This movement was clearly distinct from Brownian movement. A typical example of the condition favorable for the observation of such movement was as follows: 20 mM  $\text{MgCl}_2$ , 0.4 mg/ml F-actin, 0.13 mg/ml myosin, and 23 mM KCl in buffer A. Tropomyosin seemed to increase the probability of movement. The increases in the concentration of KCl and the molar ratio of actin to myosin were favorable. Under this condition, superprecipitation did not occur immediately after mixing actin and myosin (13), and very small precipitates appeared gradually. Around those small precipitates, dispersed myosin filaments gathered gradually together with F-actin. In such areas around the precipitates where proteins were locally concentrated, many myosin filaments were frequently observed to move ( $\sim 60$  filaments in  $900 \mu\text{m}^2$  for 15–20 s).

A myosin filament moving in the medium could frequently

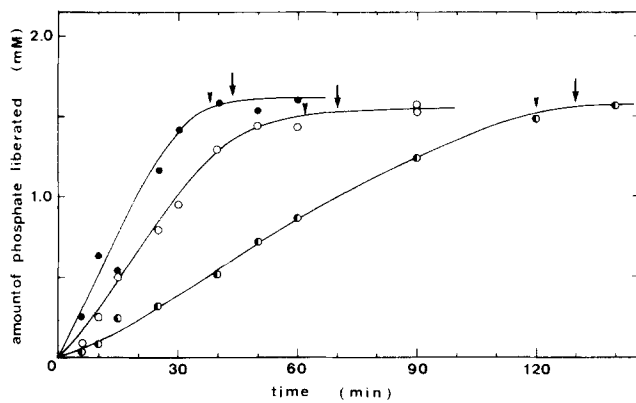


FIGURE 7 ATP consumption during the movement of myosin filaments. Phosphate liberation in the actomyosin solution was followed with time. The amount of phosphate liberated (ordinate) was shown as the concentration in the solution. At the final stage of reaction, all ATP molecules in the solution (1.5 mM) were consumed. ATP was hydrolyzed at nearly a constant rate during active movement of myosin filaments. Until the time indicated by an arrowhead associated with each line, the moving myosin filaments were found under dark field microscopy. An arrow after the arrowhead points to the time when all movements including Brownian movement of myosin filaments stopped. The medium condition was as follows: 30 mM Tris-HCl pH 7.6, 1.5 mM ATP, 21 mM MgCl<sub>2</sub>, 1 mM EGTA, 25  $\mu$ M CaCl<sub>2</sub>, and either 0.28 mg/ml F-actin and 0.4 mg/ml myosin (●), 0.15 mg/ml F-actin and 0.4 mg/ml myosin (○), or 0.28 mg/ml F-actin and 0.2 mg/ml myosin (◐) at 21°C.

change direction without rotation and intermittence of the movement (Fig. 8, right). There was no difference in the speed between forward and backward movement with respect to the filament axis of myosin ( $6.0 \pm 0.75 \mu\text{m/s}$  as shown in Fig. 6b).

### Electron Microscopy

In electron microscopy of actomyosin which shows vigorous sliding movement of myosin filaments, bundles composed of F-actin filaments were often found on a grid, in addition to many dispersed myosin and F-actin filaments. Several myosin filaments were frequently associated with the bundle, as shown in Fig. 9a. Enlarged photographs reveal myosin cross-bridges attached to F-actin, as shown in Fig. 9, b, c, and d. It is difficult to infer the direction of movement of myosin filaments along the bundle from the morphological aspects of cross-bridges which seemed to be attached to F-actin in both sides of the central bare zone. Short myosin filaments not attached to F-actin showed an apparent bipolar structure (Fig. 9e).

In the rigor state, some myosin filaments were incorporated in F-actin bundles (Fig. 10a). When these bundles were decorated with muscle HMM, arrowheads in the bundle usually pointed in the same direction as shown in Fig. 10b. A few filaments having an opposite polarity were sometimes included in the bundle. These F-actin filaments pointing in the opposite direction might be attached to the bundle after depletion of ATP during overnight storage in the cold. The degree of polarity observed was 0.7 (Fig. 11). In other words, 85% of the population pointed in the same direction. By electron microscopy, the length distribution of myosin filaments was measured as shown in Fig. 12. The average length was  $0.62 \mu\text{m}$ .

### DISCUSSION

In this paper, we described the unidirectional sliding movement of myosin filaments along the F-actin bundle which had a polar structure (Figs. 10 and 11). The unidirectionality of movement must be based on the structural polarity of the bundle, since myosin filament has a bipolar structure and the potential to move in both directions. Actually, a myosin filament moved in both directions of its long axis in the medium. The most reasonable interpretation of the results is that the myosin filament can move not only along the F-actin bundle but also along the individual F-actin filaments dispersed in the medium, and it changes the direction when it changes the track from one F-actin filament to another with opposite polarity.

The unidirectional sliding of myosin filament along F-actin bundles was reconstituted purely with actin and myosin from muscle. This movement is reminiscent of organelle transport or cytoplasmic streaming found in many kinds of cells, particularly in *Nitella* cells. If organelles were linked by myosin, the organelles would be transported along F-actin filaments. If a meshwork in the cytoplasm would be associated with myosin, the cytoplasmic streaming could occur along actin cables as suggested for the cytoplasmic streaming in *Nitella* cells (24). In our reconstituted system, a bulk flow of the medium did not occur, although it was reported in narrow capillaries (33) or in an artificially produced apparatus that mimicked the cell (37, 38). To produce the bulk flow, a highly organized system would be required.

In the medium used here, F-actin usually formed Mg-paracrystals that dispersed immediately after myosin was added. Fragmentation of F-actin by HMM in the presence of ATP (36) suggests a strong force generated by the interaction between F-actin and myosin or HMM with ATP. Dispersion of Mg-paracrystal may be caused by an interaction that makes unstable the antiparallel arrangement of F-actin in Mg-paracrystal (7). For formation of the bundle with a polar structure, the interaction between F-actin and myosin in the presence of ATP seems to be important. In the case of actomyosin from *Physarum*, the bundles formed after superprecipitation also have polarity (22).

Since the force generated by the interaction between F-actin and myosin acts on both F-actin and myosin filaments, short bundles of F-actin floating freely in the medium are expected to move. Nevertheless, we did not observe the movement of those bundles. There are a lot of dispersed F-actin filaments forming a meshwork in the medium, and the observable bundles are not free but are mostly parts of the meshwork. Therefore, the bundles are stationary, while many myosin filaments slide successively on the bundle.

The maximum velocity of the sliding of myosin filaments along the bundles or dispersed F-actin filaments is  $\sim 10 \mu\text{m/s}$  (Fig. 6). Previously, we reported that the maximum velocity of travel for fibrils isolated from *Nitella* was  $20 \mu\text{m/s}$  (11) and that of movement for fibrils composed of actin and myosin filaments from muscle was  $\sim 30 \mu\text{m/s}$  at room temperature (12). These values obtained in *in vitro* systems are of the same order as the maximum shortening velocity of a half of a sarcomere in skeletal muscle (i.e.,  $5 \mu\text{m/s}$  in glycerinated rabbit psoas at 20°C [35], and  $13 \mu\text{m/s}$  in frog sartorius at 22°C [9]). The fact that a short myosin filament  $0.6\text{-}\mu\text{m}$  long (Fig. 12) slides at an average velocity of  $6 \mu\text{m/s}$  (Fig. 6) raises a serious question as follows.

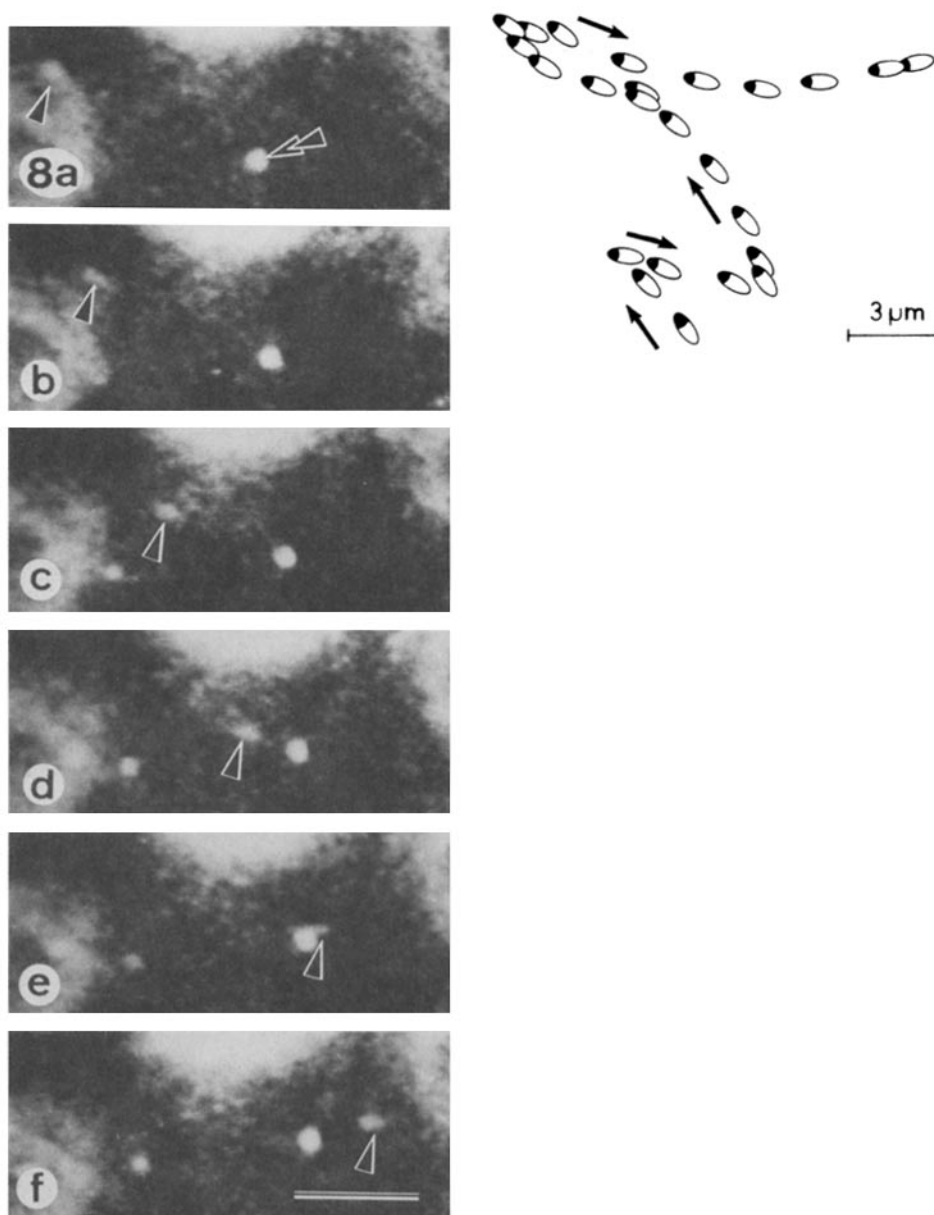


FIGURE 8 Myosin movement along dispersed F-actin filaments. (Left) A myosin filament indicated by an arrowhead moved actively along a dispersed, perhaps a single, F-actin filament that was invisible with a dark field microscope. A small particle indicated by a double arrowhead in a moved slowly from left to right due to the passive flow of the medium. The medium condition was as follows: 24 mM  $MgCl_2$ , 0.4 mg/ml F-actin, 0.05 mg/ml tropomyosin, 0.2 mg/ml myosin, 10 mM KCl, and the others were the same as for Fig. 2. The time interval between successive photographs was  $\frac{1}{2}$  s. Bar, 5  $\mu m$ .  $\times 3,300$ . (Right) Illustration of the track of the movement of a myosin filament along dispersed F-actin filaments. A myosin filament was traced as an ellipse, a particular side of which was marked with black color to show the reverse movement. The movement was reversed three times in this case, suggesting that the myosin filament could move in reverse, when it changed the track of F-actin filament. The movement after the last change of the direction is shown in the left-hand panel. Each ellipse was depicted every  $\frac{3}{8}$  s.

Sliding of myosin filaments along F-actin bundles is observed at low ionic strengths. According to recent biochemical investigations, the interaction of myosin with F-actin at low ionic strengths is characterized by long term binding of myosin to F-actin and ATP hydrolysis without dissociation of myosin from F-actin (18, 31, 35). Such an interaction would be important for the sliding movement of myosin filaments along the F-actin bundle from one end to the other. For myosin filaments to slide along F-actin filaments, it is necessary to keep contact with F-actin and interact consecutively with actin molecules arrayed in the F-actin filament by repeating the cycle of ATP splitting.

Based on structural analysis of a myosin filament, myosin heads project in the same radial angle at a repeating distance of  $14.3 \times 3$  nm (43 nm) (17, 30). If, geometrically, only these cross-bridges located in half of a myosin filament could attach to an F-actin filament, a short myosin filament 0.6- $\mu m$  long has only 5–6 cross-bridges available to generate the effective sliding force when interacting with a single F-actin filament.  $[(600 - 150) / (43 \times 2)] = 5.2$ , assuming the central bare zone is 150 = nm long [16]. If myosin molecules could attach to an F-actin filament at most every 14.3 nm, the number of attached cross-bridges per half myosin filament becomes 15.

Biochemical measurements gave a value of 8.3  $s^{-1}$  per

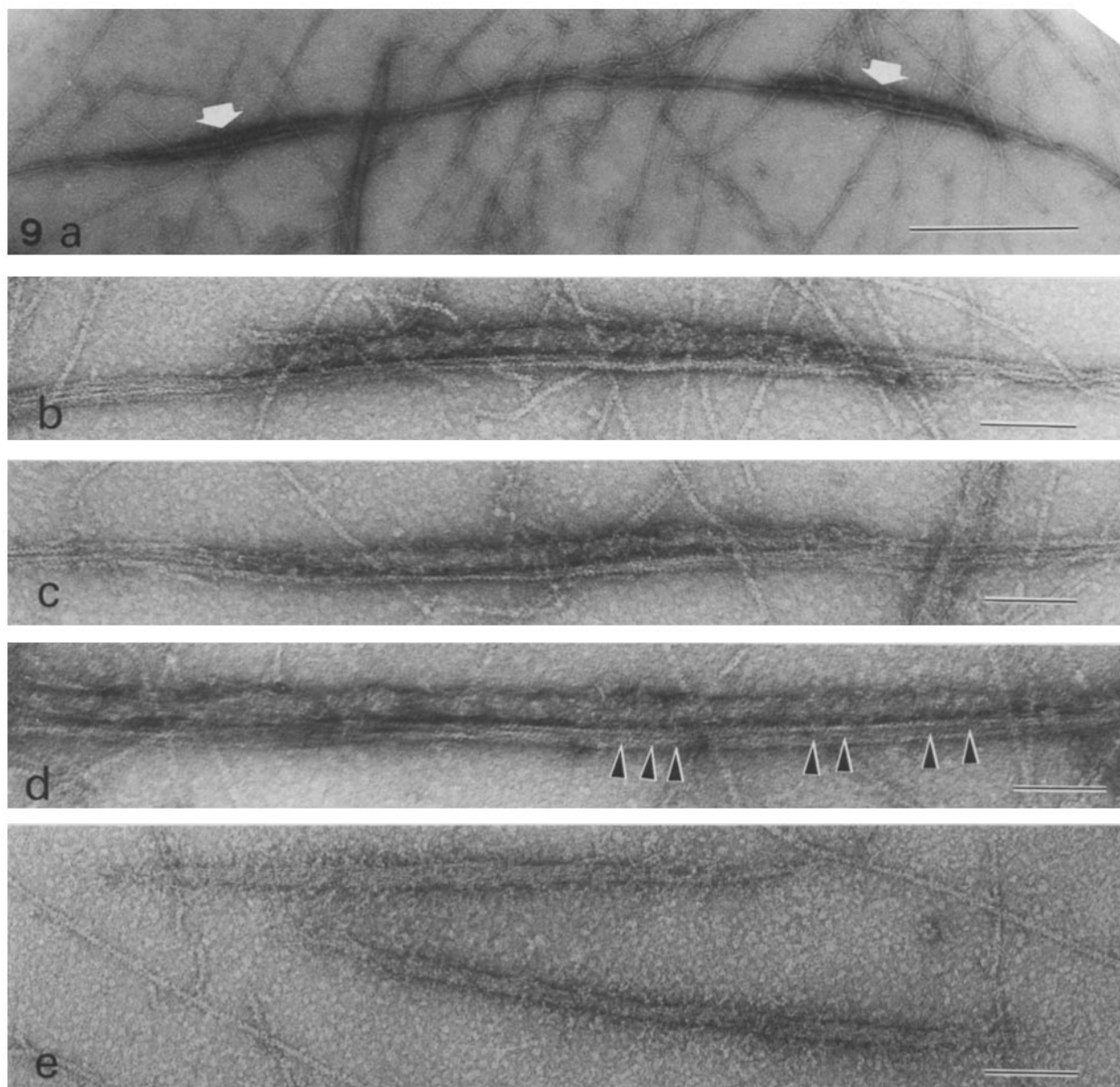


FIGURE 9 Electron microscopy of actomyosin during the active movement. (a) Myosin filament attached to a bundle of F-actin filaments (indicated by arrows). Bar,  $0.5 \mu\text{m}$ .  $\times 50,000$ . (b and c) Myosin filaments indicated by arrows in a are enlarged. Bar,  $0.1 \mu\text{m}$ .  $\times 140,000$ . (d) Another example of myosin filament attached to a bundle of F-actin filaments. Cross-bridges (arrowheads) are clearly visible in the right half of the myosin filament in the figure. Bar,  $0.1 \mu\text{m}$ .  $\times 140,000$ . (e) Myosin filaments not attached to F-actin filaments found on the same grid. Myosin filaments were tapered at both ends. Bar,  $0.1 \mu\text{m}$ .  $\times 140,000$ .

myosin head for the maximum rate of actin-activated ATPase of myosin at the extreme of a high concentration of F-actin at low ionic strength without dissociation of myosin from F-actin at room temperature (18). In rabbit skeletal muscle, the rate constant was evaluated as  $1.5 \text{ s}^{-1}$  per myosin head for the rate limiting process during the cross-bridge cycle (8). If the biochemical datum of  $8.3 \text{ s}^{-1}$  per myosin head was applied to the ATP hydrolysis in myosin filament sliding along F-actin, the  $0.6\text{-}\mu\text{m}$ -long myosin filament hydrolyzes  $6 \times 2 \times 8.3 = 100$  ATP molecules every second ( $18 \times 2 \times 8.3 = 300$ , at most), on the assumption that a myosin molecule has two identical heads. Therefore, in order that myosin filament slides at  $6 \mu\text{m/s}$ , the hydrolysis of one ATP molecule must result in the sliding of  $60 \text{ nm}$  ( $20 \text{ nm}$  at least) of a myosin

filament. This distance of sliding per ATP hydrolysis is much longer than one stroke of myosin cross-bridge which is assumed to be  $\sim 10 \text{ nm}$  in the current sliding theory (14, 15). Conversely, if a myosin cross-bridge advances  $10 \text{ nm}$  by the hydrolysis of one ATP molecule, a short myosin filament  $0.6\text{-}\mu\text{m}$  long must hydrolyze ATP at a rate of  $6,000/(10 \times 12) = 50 \text{ s}^{-1}$  per myosin head. Thus, the high velocity of sliding of short myosin filaments observed here raises the problem whether the rate of ATP hydrolysis during sliding is extremely high, or the hydrolysis of one ATP molecule produces sliding of extraordinarily long distance.

The reconstituted system developed by us should be very useful and informative in elucidating the molecular mechanism not only of muscle contraction, but also of cell motility based on actomyosin.



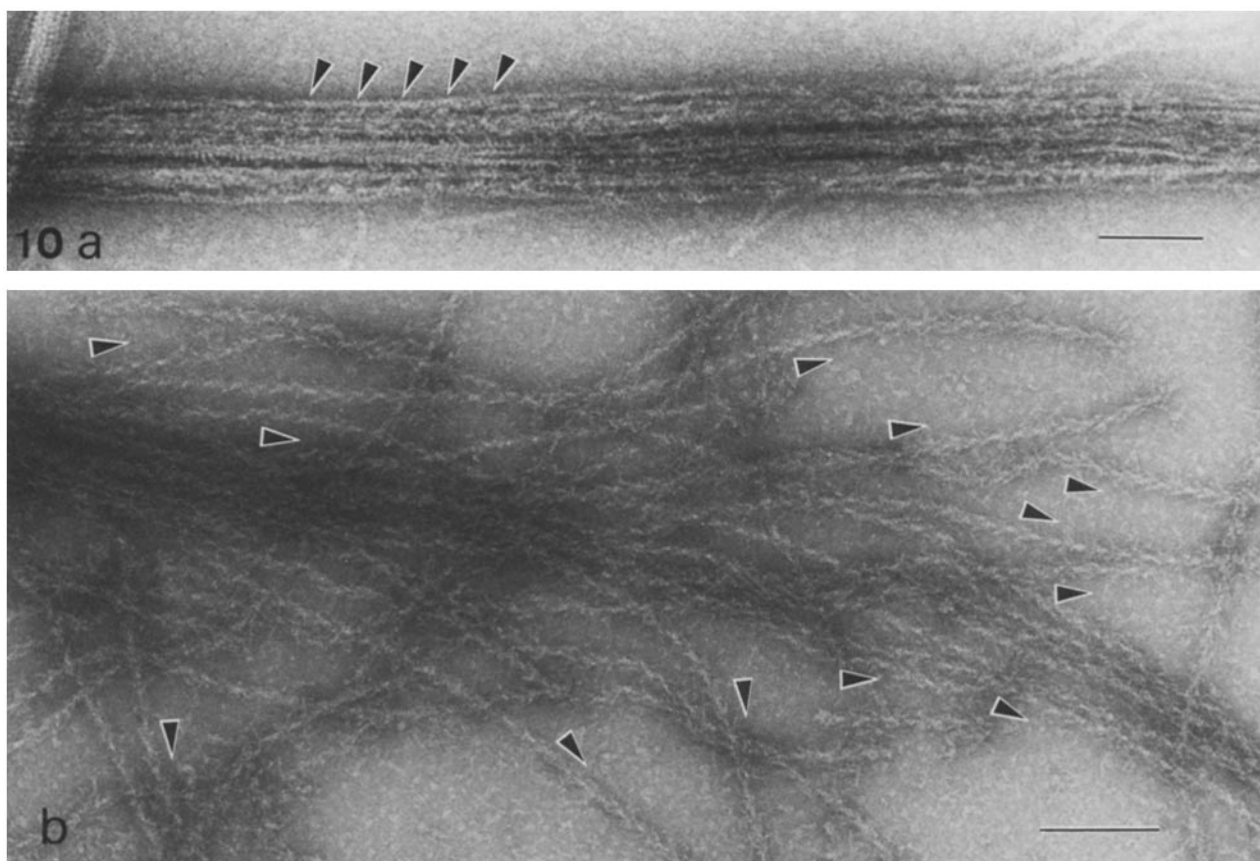


FIGURE 10 Bundle decorated with muscle HMM. (a) A bundle of F-actin filaments after exhaustion of ATP. Myosin filaments were seen to be incorporated in the bundle. Cross-bridges were seen as indicated by arrowheads in the figure. Bar,  $0.1 \mu\text{m}$ .  $\times 140,000$ . (b) Decoration of the bundle with HMM. F-actin filaments decorated by HMM (arrowheads) are pointed in the same direction in the bundle. Bar,  $0.2 \mu\text{m}$ .  $\times 80,000$ .

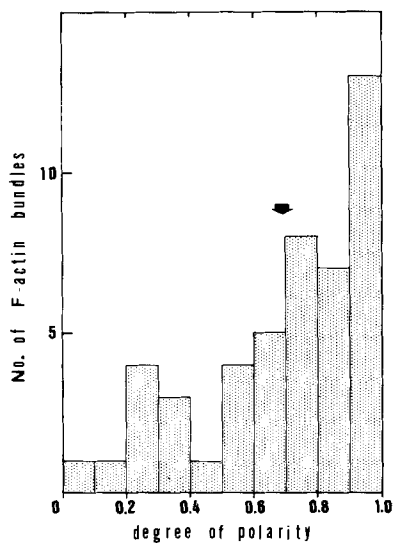


FIGURE 11 Degree of polarity of the F-actin bundle. The number of F-actin filaments whose arrowheads pointed in the same direction ( $n_1$ ) and the opposite direction ( $n_2$ ) were counted in a bundle. Total number of filaments counted in a bundle varied from 5 to 25. The degree of polarity ( $p$ ) was calculated as follows:  $p = (n_1 - n_2) / (n_1 + n_2)$ . The arrow in the figure points to the mean (46 bundles and 470 filaments were measured; mean, 0.69).

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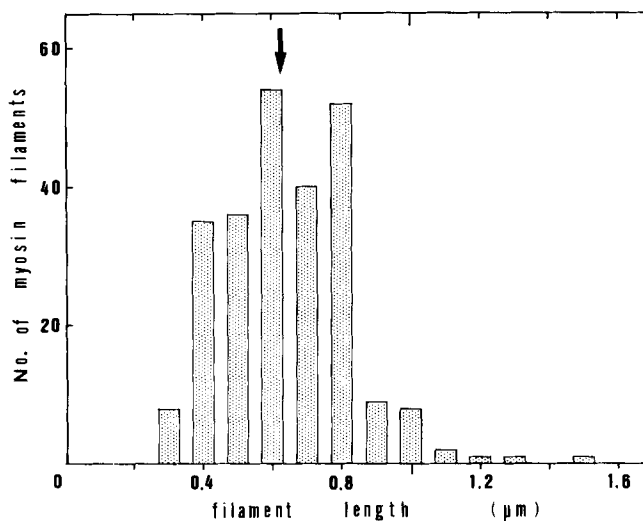


FIGURE 12 Length distribution of myosin filaments. Actomyosin showing sliding movement was observed by electron microscopy. All myosin filaments found on an electron microscopic film were measured (42–56 filaments on a film). Only a few aggregated filaments were omitted. The arrow points to the mean (246 filaments were measured; mean,  $0.62 \mu\text{m}$ ; SD,  $0.19 \mu\text{m}$ ).

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