

Active Movement In Vitro of Bundles of Microfilaments Isolated from *Nitella* Cell

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ABSTRACT Subcortical fibrils composed of bundles of F-actin filaments and endoplasmic filaments are responsible for endoplasmic streaming. It is reported here that these fibrils and filaments move actively in an artificial medium containing Mg-ATP and sucrose at neutral pH, when the medium was added to the cytoplasm squeezed out of the cell. The movement was observed by phase-contrast microscopy or dark-field microscopy and recorded on 16-mm film.

Chains of chloroplasts linked by subcortical fibrils showed translational movement in the medium. Even after all chloroplasts and the endoplasm were washed away by perfusion with fresh medium, free fibrils and/or filaments (henceforth, referred to as fibers) not attached to chloroplasts continued travelling in the direction of the fiber orientation. Sometimes the fibers formed rings and rotated. Chloroplast chains and free fibers or rings continued moving for 5–30 min at about half the rate of the endoplasmic streaming *in vivo*. Calcium ion concentrations $<10^{-7}$ M permitted movement to take place. Electron microscopy revealed that both fibers and rings were bundles of F-actin filaments that showed the same polarity after decoration with heavy meromyosin.

Since the contractile proteins actin and myosin have been found in a variety of nonmuscle cells, cell motility has been postulated to be produced by an interaction between actin and myosin. It is now significant to ask whether the force-generating process in nonmuscle cells is similar or identical with that in muscle.

Cells of the characean algae show a well-ordered cytoplasmic streaming. The hypothesis that the force for streaming is generated at the interface between motile endoplasm and stationary ectoplasm (15) stimulated the investigation of the structure of the interface. According to light and electron microscope studies, subcortical fibrils lie along the inner surface of rows of chloroplasts (13, 14) that are located at the interface between endoplasm and ectoplasm (20, 27). These fibrils are composed of bundles of thin filaments (25) which have been confirmed to be F-actin filaments (26, 31). After decoration of the fibrils *in situ* with muscle heavy meromyosin (HMM), all arrowheads point in the direction opposite to cytoplasmic streaming (19).

Another hypothesis on the mechanism of the force generation of streaming in the *Nitella* cell was postulated recently by Allen (1) and Allen and Allen (2) who discovered the endoplasmic filaments anchored on subcortical fibrils. The wave motion of the endoplasmic filaments could explain the force enough for endoplasmic streaming.

Myosin was isolated from *Nitella* recently (18). It might be

dispersed in the endoplasm (5) or attached to some organelles streaming on the fibrils (23). The treatment of ectoplasm with cytochalasin B or D inhibited the streaming (24). The interaction between F-actin at the interface and myosin in endoplasm may induce streaming, which requires Mg-ATP (28, 32).

Physiological approaches at the subcellular level also have been tried for these two decades. Rotation of fibrous polygons and chloroplasts has been observed in endoplasmic droplets isolated from the cell (8–11, 16, 21). The movement of chains of chloroplasts detached from ectoplasm has been found in a whole cell subjected to instantaneous acceleration (17). However, the movement of these polygons or chloroplasts cannot easily be controlled artificially as long as the droplet or the cell retains a membrane.

Kuroda and Kamiya (22) succeeded in the preservation of rotation of isolated chloroplasts in an artificial medium and partial reactivation of their rotation by muscle HMM. The control of motility was also examined in isolated cytoplasm from amoeba (29). This line of investigation is now very promising for the elucidation of the molecular mechanism of cell motility, because movement may be controlled by changing the composition of the medium.

From this point of view, I have tried to induce cytoplasmic fibrils isolated from *Nitella* to move actively in an artificial medium. In fact, translational and rotational movements of

fibers have been observed *in vitro*, which may give us useful suggestions on the mechanism of cell motility.

MATERIALS AND METHODS

Cultivation of Nitella

Nitella microcarpa Braun was cultivated in a polyethylene bucket filled with 40 liters of water containing ~10 g of commercial plant food (Hyponex, by the Hyponex Co. Inc., Copley, Ohio), at a temperature of 20°C under illumination of a fluorescent lamp lighted during the daytime. Under suitable conditions, plants grew by two to three internodes a week. Internodal cells of ~4 cm in length were used.

Preparations of Specimens for Light Microscopy

One end of an internodal cell of *Nitella* from near the apical end was excised, and the whole cell content was squeezed and mounted on a glass slide. 5–10 vol of an activating medium was added to the isolated cytoplasm. The specimen was covered with a coverslip and then observed with a light microscope. The composition of the activating medium was as follows; 1.5 mM ATP, 2 mM MgSO₄, 0.2 M sucrose, 4 mM EGTA, 0.1 mM CaCl₂, 60 mM KCl, and 10 mM imidazole buffer of pH 7.0. This medium and modifications of it were used through the course of the experiments.

For dark-field microscopy, a large number of chloroplasts floating in the medium were washed away by perfusion with fresh activating medium. The effect of some chemicals was also examined by perfusion with the medium containing those chemicals. All preparations and observations were performed at room temperature.

Light Microscopy and Cinematography

Chains of chloroplasts were observed with a phase-contrast microscope (Olympus model FHT, Olympus Kogaku Inc., Tokyo, Japan). Fibers not attached to chloroplasts were observed with a dark-field microscope equipped with a mercury lamp (Ushio Electric Inc., Japan; type USH 102D) and an Olympus apochromatic objective Apo 40 X, NA: 1.0 (7). Movements of those chloroplasts and fibers were recorded on 16-mm films (Kodak plus X negative for phase contrast microscopy and Kodak 4 X negative for dark-field microscopy) with a Bolex camera at 16 frames per second.

Electron Microscopy

The specimen was first monitored by a phase-contrast microscope. If a lot of chains of chloroplasts were moving, the coverslip was removed with care and a drop of green suspension of chloroplasts was mounted on a carbon-coated collodion or Formvar film on a grid. After a few minutes, the specimen was briefly rinsed with the fresh medium and stained with 1% uranyl acetate.

The following process was necessary to attach rotating rings to a film: One to three grids were first attached to a glass slide with Borden mesh cement (Ohken Shoji, Japan). A drop of squeezed cytoplasm was mounted on the grid and the activating medium was added. The subsequent procedure for preparation was the same as that described above.

Cytoplasmic fibrils were decorated with HMM according to the following procedure: Before staining, the specimen was washed with the activating medium from which ATP was omitted and then the medium containing HMM was poured on the grid. After a 2-min incubation, excess HMM was washed away with the medium devoid of ATP and then the specimen was stained.

Specimens were viewed with an electron microscope (JEM 100-C) operating at 80 kV.

RESULTS

Activating Medium

The cytoplasm squeezed out of the cell contained endoplasm, ectoplasm, chloroplasts, and contents of vacuoles. The endoplasm formed droplets enclosed by membranes. Almost all chloroplasts lay outside of the droplets and did not show any movement. After the addition of the activating medium to the cytoplasm, the membrane of the endoplasmic droplet was broken and the endoplasm dispersed into the medium, then fibers and chains of chloroplasts began to move. In some

preparations, almost all chloroplasts moved in the activating medium.

The movement of fibers or chloroplast chains required 0.2–0.3 M of sucrose and >1 mM of Mg-ATP. At a concentration of ATP <1 mM, the membrane of the endoplasmic droplets was not disrupted and fibers or chains of chloroplasts did not move. Apparently, some components in the endoplasm were necessary for the movement of fibers or chloroplast chains *in vitro*. When MgSO₄ was omitted, the movement of chloroplast chains was not discernible by phase-contrast microscopy, but by dark-field microscopy a few very short fibers not attached to chloroplasts were observed moving about one hundred times more slowly than in the standard activating medium. The movement of fibers and chloroplast chains was not affected by omitting CaCl₂ or KCl or both, by changing the pH from 7.0 to 8.0, or by substituting Tris-HCl, Tris-maleate, or potassium phosphate buffer for imidazole buffer. The movement was activated markedly by the addition of EGTA. The duration of the movement was not prolonged by the addition of 1 mM of dithiothreitol or polyethylene glycol (0.5%) or both to the medium.

Behavior of Chloroplast Chains

The activating medium induced chains of chloroplasts linked by cytoplasmic fibrils to move as shown in Figs. 1 and 2. Although the length of the chain varied from a single chloroplast to a long chain of about 30 chloroplasts, the speed of movement did not depend on the length; it was ~10 μm/s, the same order of the velocity of cytoplasmic streaming in the living cell (40 μm/s). Chains did not move straight but curved and turned irrespective of their lengths. A short chain of chloroplasts in Fig. 2 displayed rotatory movement after the posterior end of the chain was attached to the substratum. Such behavior of chloroplast chains showed that the movement was active and that the driving force was generated in all parts of the chain.

The active movement of chains diminished gradually, not only in speed but also in the number of moving chains, and finally stopped within 5–10 min. After chains stopped moving, they were not reactivated by perfusion of fresh medium, or by mechanical agitation to detach chloroplast chains from the substratum.

Not all chloroplast chains showed active movement. There were apparently two kinds of chains: motile and nonmotile ones. Motile and nonmotile parts could coexist even in a single chain. A chain in the upper right portion of Fig. 1 *e–h* shows such an example. The anterior part of ~10 chloroplasts in this chain lost the ability to generate a force, so that it was dragged by the posterior part that continued moving. The moving posterior was taut, but the dragged anterior was slack as indicated by an arrow in Fig. 1 *h*. Another example was a long chain in Fig. 1 *a–d*. One of two long moving chains that separated in the middle of the photograph, turned around actively downward, and then the anterior part lost the ability to move and was passively rounded up by the advancement of posterior part. Another remarkable phenomenon was that some of the stationary chains suddenly started to move. For example, a chain in the lower right of Fig. 1 *a* and *b* did not move at first, but then began to move as shown in Fig. 1 *c–e*. These facts suggested that some control mechanism switched the state of cytoplasmic fibers between an active state generating a force and an inactive state not generating any force.

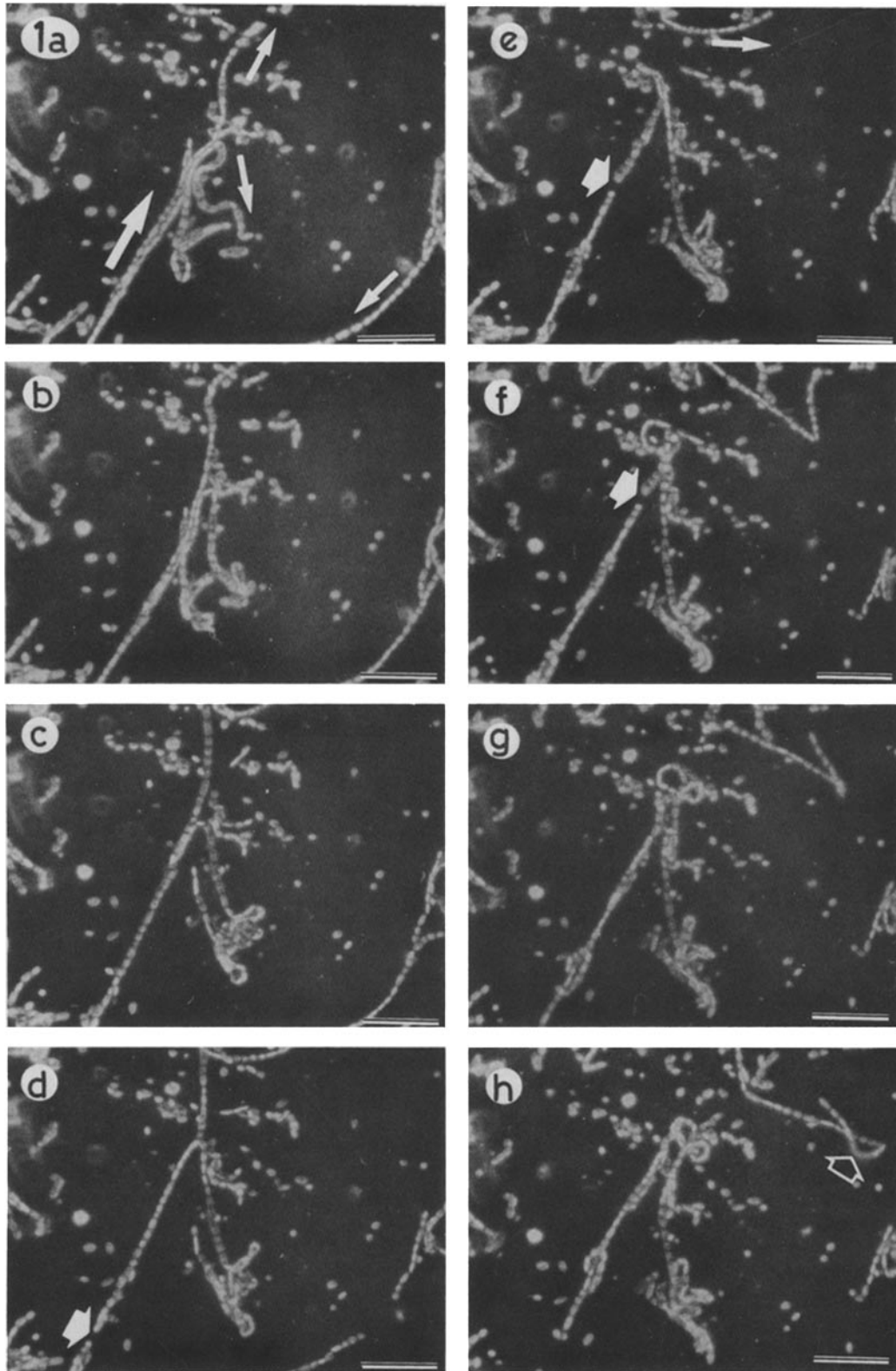


FIGURE 1 In vitro movement of long chains of chloroplasts. Successive photographs taken with a phase-contrast microscope at the time interval of 12 s show the same area of the specimen. Three long chloroplast chains were moving at $\sim 10 \mu\text{m/s}$. The direction of movement is indicated by arrows in *a* and *e*. In the middle of each photograph, one of the long chains turned downward and rounded up. The other chain in the middle of the photograph moved upward. The anterior half of this chain continued moving and changed direction, as indicated by the arrow in *e*, but between *e* and *f*, several chloroplasts at the top of the chain stopped active movement and were passively pulled by the posterior part, as indicated by the arrow in *h*. The movement of a gap in the array of chloroplasts can be followed in the photographs as shown by short arrows in *d-f*. Bar, $50 \mu\text{m}$. $\times 230$.

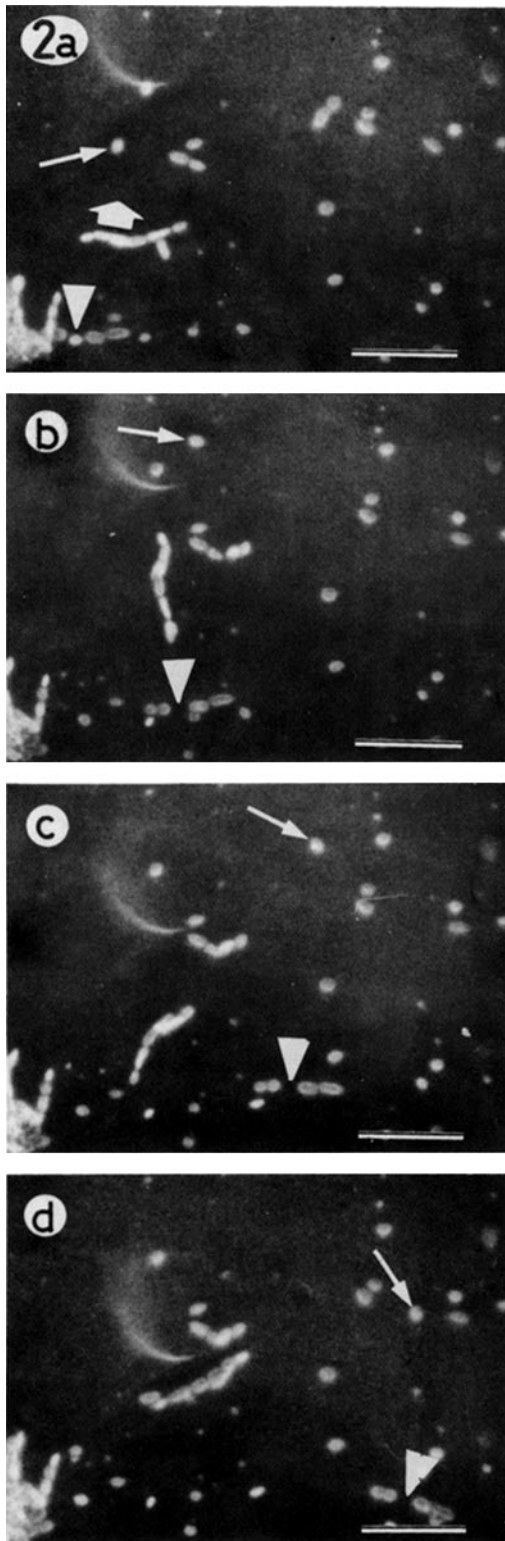


FIGURE 2 In vitro movement of single chloroplasts and short chains of chloroplasts. Successive photographs were taken with a phase-contrast microscope at time intervals of 8 s. A long arrow in each photograph indicates the movement of single chloroplast. A white arrowhead indicates a stretched part of a chain of four chloroplasts. A short arrow in *a* indicates the direction of rotatory movement of a chain. This rotatory movement was caused by attachment of one end of the chain to the substratum. Bars, 50 μm . $\times 270$.

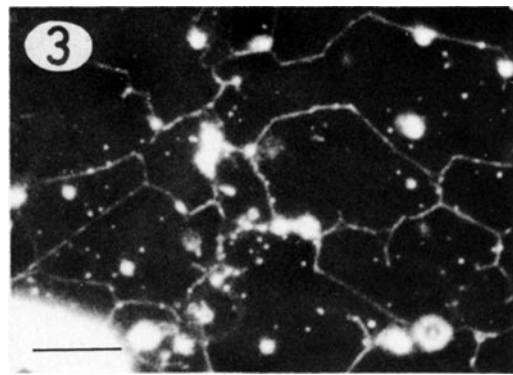


FIGURE 3 Network fibers by dark-field microscopy. The network fibers in the photograph spread widely and attached to the glass surface at several points. They were probably formed when a coverslip was applied to the isolated endoplasm. They were extended largely by the flow of the medium. In electron micrographs of negatively stained specimens, they looked membranous and did not show any filamentous structure like F-actin. Bar, 10 μm . $\times 1,150$.

Rotating Rings

Fibers not attached to chloroplasts could be observed by dark-field microscopy. The movement of the fibers was not disturbed by the flow of the medium to wash floating chloroplasts away. The movement was observed mostly within an area very close to the surface of the glass slide. From this fact, the fibers might have some interaction with the glass surface.

The fibers not attached to chloroplasts often formed rings and showed active rotation. Rotating rings observed here would be the same as that observed first by Jarosch in an endoplasmic droplet (8, 10). In his case, fibers made polygons or occasionally circles, and the movement of polygons was classified into two types, rotatory and undulatory (13). In my preparation, however, the fibers showed rotation exclusively. Their shapes were mostly circular, rarely polygonal.

Most rings and polygons were a few micrometers in diameter. Rotation speed was usually 1–3 rps. The direction of rotation did not reverse, suggesting the polarity of the fibers of the ring (see section entitled Electron Microscopy). In careful analyses of the film, only a single ring was found to change its direction of rotation alternatively. However, this ring actually consisted of two rings, each of which probably had a different polarity.

The rotation of rings continued for ~ 30 min and slowed down gradually and then stopped. The rotatory movement did not change into undulations.

Rotating rings could attach to small granules or chloroplasts. Sometimes, the rings attached to fibers of a network, which was spread widely on the glass slide, and did not show any active movement except Brownian motion in the activating medium (Fig. 3). When network fibers or granules were attached tightly to the rotating ring, they rotated at the same speed as rotation of the ring (Fig. 4). On the other hand, the attachment of the network fibers in Fig. 5 or the chloroplast in Fig. 6 to the rotating ring was not tight. The network fibers or the chloroplast interacted weakly with the ring. The network fibers in Fig. 5 did not rotate in accordance with rotation of the ring but slipped on the ring. The rotating ring in Fig. 6 is the same one as in Fig. 5. The chloroplast rotated around the rotating ring at a speed of 0.3 rps while the ring continued rotating at a constant speed of 2.7 rps. Granules and chloro-

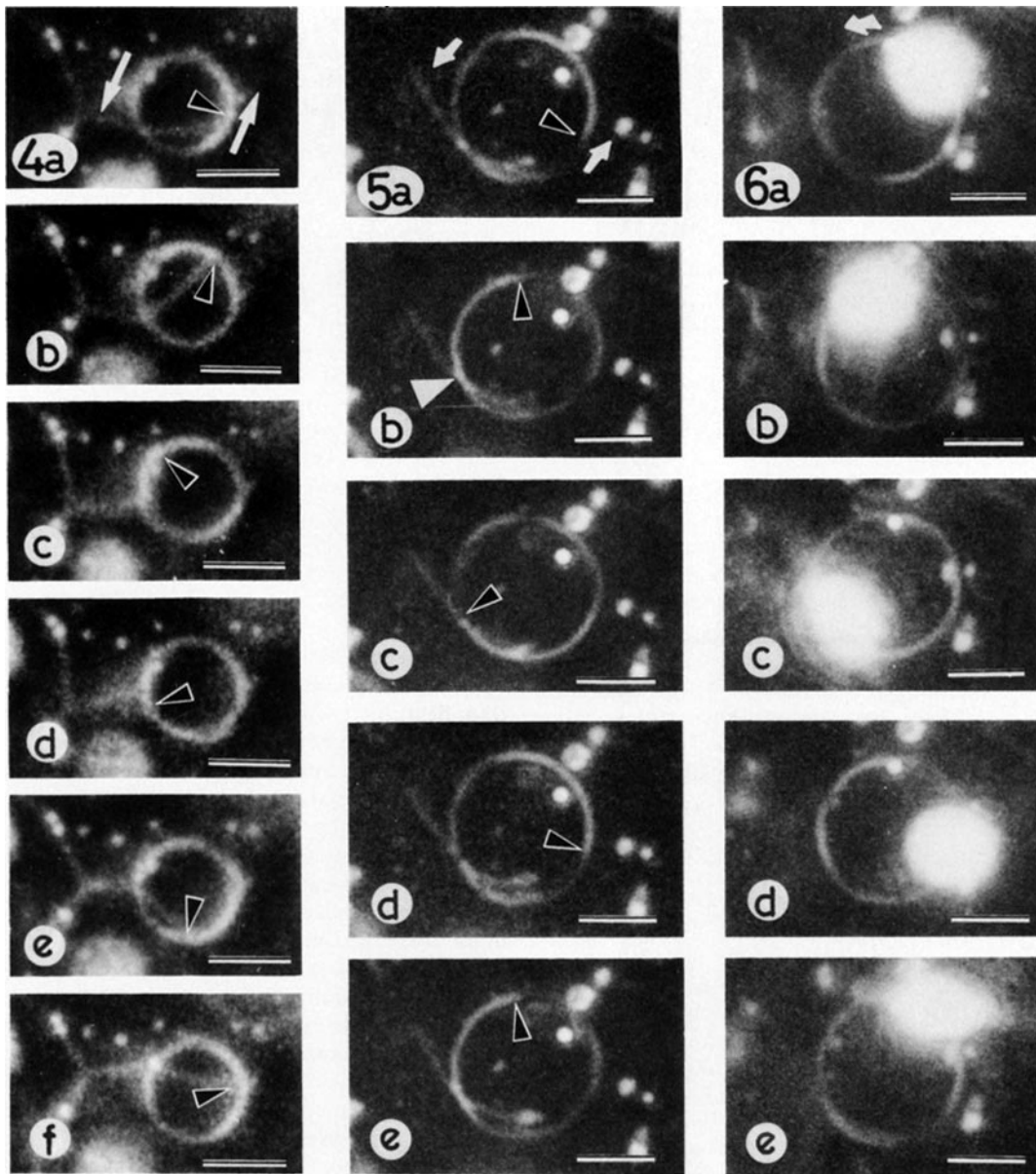


FIGURE 4 A rotating ring with a network fiber attached. Successive photographs were taken at time intervals of $\frac{1}{8}$ s. The direction of rotation of the ring is indicated by the arrows in *a*. An end of a thin network fiber was attached to the ring at a point indicated by the arrowheads. The attached end rotated with the rotation of the ring and the thin fiber was stretched by the rotation. Bars, $5\ \mu\text{m}$. $\times 2,200$.

FIGURE 5 A rotating ring under a dark-field microscope. Successive photographs were taken at time intervals of $\frac{1}{8}$ s. The direction of rotation of the ring is indicated by the white arrows. A black and white arrowhead in each photograph points to a particular point fixed on the rotating ring. The speed of rotation was ~ 2.7 rps. A network fiber was loosely connected with the rotating ring as shown by a white arrowhead in *b*. The connection slipped on the ring. Bars, $5\ \mu\text{m}$. $\times 2,100$.

FIGURE 6 Rotation of a chloroplast attached to a rotating ring. The rotating ring in this photograph is the same one as in Fig. 5. A chloroplast approached and finally attached to the ring and began to rotate with the ring. The white arrow shows the direction of the rotation of the ring and the movement of the chloroplast. Successive photographs were taken at time intervals of 1 s. The chloroplast rotated at a speed of $\frac{1}{8}$ rps, 13 times slower than the rotation of the ring. Bars, $5\ \mu\text{m}$. $\times 2,100$.

plasts weakly attached to the rotating ring moved always in the same direction as the rotation of the ring.

The fiber of the rotating ring was taut, but when the ring stopped rotation, it was sometimes deformed and made concave as shown in Fig. 7*b*. This phenomenon suggested that the fiber lost its tension and became slack when it was not able to

generate any force, as already described in the preceding section.

Travelling Fibers

When the fiber did not form a ring but had free ends, it showed translational movement in the direction of the fiber, at

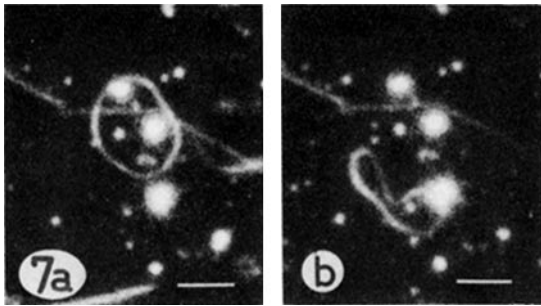


FIGURE 7 An example of loss of tension in a nonmotile fiber. (a) A rotating ring. (b) The ring shown in a became concave when it stopped. Bars, 5 μm . $\times 1,400$.

a speed of $\sim 20 \mu\text{m/s}$ (Fig. 8). The translation was unidirectional and never reversed. These "travelling" fibers were not straight but mostly curved. The curvature was not fixed but varied. The direction of movement also changed along the fiber as if each part of the fiber generated a force in the tangential direction.

Some travelling fibers had a few chloroplasts, suggesting that the travelling fibers were derived from the cytoplasmic fibrils originally associated with chloroplast chains. After most of the chloroplasts were removed, the cytoplasmic fibers continued to show active movement as the rotating rings did. Some travelling fibers were spontaneously converted into rotating rings (Fig. 9). This means that rotating rings were derived also from the same kind of cytoplasmic fibrils. The speed of translational movement of the travelling fibers was almost the same as the speed of rotation of the rings. It was reported previously that the subcortical fibrils could be converted into rotating polygons in a centrifuged *Nitella* internode (13).

Effect of Ca^{2+} on Rotating Rings

The rings continued rotation in the same field on a glass slide and the rotation was not disturbed by the flow of the medium. Therefore, the effect of Ca^{2+} on the rotation was conveniently examined by perfusion of the medium containing various concentrations of Ca^{2+} . The concentration of free Ca^{2+} in the activating medium was roughly estimated to be 2×10^{-8} M, assuming the association constant of Ca^{2+} with EGTA of 4.8×10^6 at pH 7.0 (4).

The removal of free Ca^{2+} from the medium was favorable to the rotation of rings. The speed of rotation was gradually decreased with increasing concentration of free Ca^{2+} , although the rotation did not stop until the concentration reached 1 mM. Upon addition of millimolar calcium ions, some rings stopped immediately while some others rotated very slowly for a while before they stopped. If Ca^{2+} was removed before cessation of the rotation, the speed of rotation was fully recovered. However, once the rings stopped, they attached tightly to the glass surface and the rotation could not be revived by the removal of Ca^{2+} .

Electron Microscopy

The cytoplasmic fibers connecting chloroplast chains, rotating rings, and travelling fibers were all composed of bundles of microfilaments (Fig. 10a and b, Fig. 11a and b). Electron micrographs showed that the microfilaments were actually F-actin and, after decoration with HMM, all arrowheads pointed in the same direction in each bundle (Figs. 10c and 12). The alignment of F-actin filaments in the bundle was slightly

disturbed by the decoration with HMM. The unidirectionality of movement can be attributed to this structural polarity. The travelling fibers were convertible to rotating rings. In electron micrographs, both fibers, travelling and rotating, gave pictures of bundles of F-actin filaments.

DISCUSSION

In the living cell, endoplasmic streaming has been postulated to be produced by a shearing force generated at the interface between ectoplasm and endoplasm by an interaction of microfilament bundles on the inner surface of the ectoplasm with some components in the endoplasm (15). According to this model, if the subcortical fibrils were not fixed but free in the endoplasm, the shearing force would result in the translational motion of the fibrils. The cytoplasmic fibers isolated here were confirmed to consist of F-actin filaments oriented in parallel with the same polarity, just as the fibrils previously found at the interface between ectoplasm and endoplasm (19). Therefore, it is likely that the travelling of fibers and the rotation of rings observed *in vitro* were produced by the same force-generating mechanism as the endoplasmic streaming *in vivo*. The fact that the rotation of chloroplasts *in vitro* was reactivated by muscle HMM (22) encourages us to believe that the interaction between actin and myosin is the fundamental mechanism of the travelling of the fiber and the cytoplasmic streaming.

However, Allen (1) and Allen and Allen (2) have recently proposed another hypothesis based on their observation that the force for streaming can mainly be generated by the wave propagation along the endoplasmic filaments branched from subcortical fibrils. This motion, like a flagellar motion, would be more convenient for understanding the travelling of fibers. Because my preparation started with the whole cell contents, both subcortical fibrils and endoplasmic filaments may be contained in the sample. However, any wavy motion of travelling fibers and rotating rings was not observed by dark-field microscopy. The curvature of the fiber changed only during a change of the travelling direction.

Among various models proposed for the mechanism of nonmuscle cell motility, an idea proposed by Tilney (30) that alteration of the packing of microfilaments generates acrosomal movement is interesting. But this idea is not readily applicable to the present case, because the acrosomal reaction is a transient process and not a continuous one. The cycle of contraction and relaxation of cytoplasm which was proposed by Allen and Taylor (3) and Taylor et al. (29) as a mechanism of amoeboid movement has not been observed in characean cells. The twisting F-actin filament model for the movement of fibers in endoplasmic droplets proposed by Jarosch (12) may be useful to understand the undulation of polygons; however, it is not suitable to explain the travelling of free fibers.

Hereafter, I will speculate on the mechanism of the travelling of fibers and rotation of rings, based on the sliding filament model of muscle contraction.

Myosin from muscle is insoluble at low ionic strength but it is quite soluble in the activating medium used here, and myosin threads are not found by dark-field microscopy (data not shown). Therefore, if *Nitella* myosin was involved in the travelling of fibers, it would be mostly in a monomeric state and repeat association and dissociation with F-actin in the presence of ATP. According to the sliding filament model, myosin molecules or the cross-bridges would undergo a conformational change on F-actin filaments, like oars of a row boat, to produce

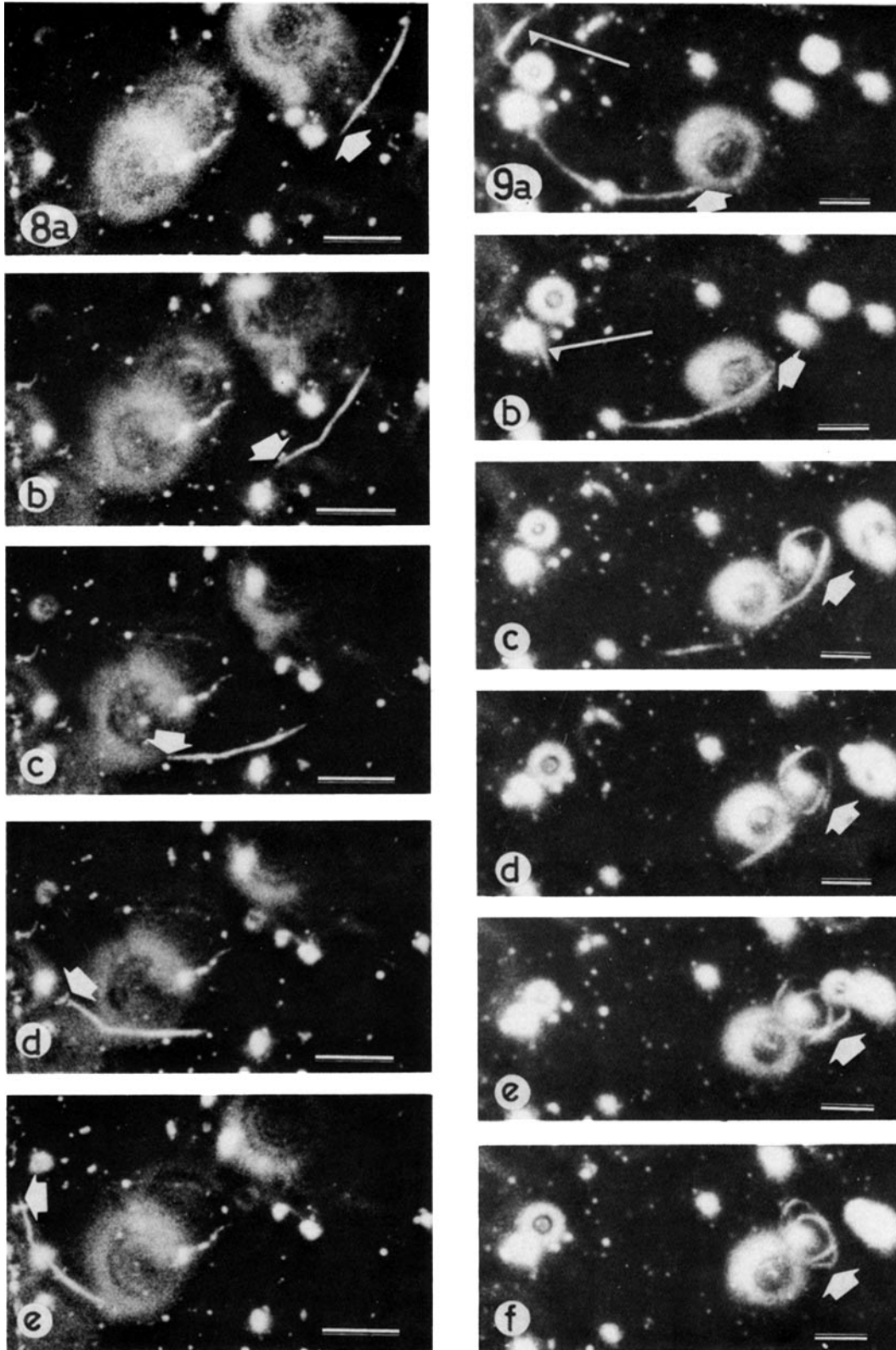


FIGURE 8 A travelling fiber observed with a dark-field microscope. Successive photographs were taken at time intervals of 1 s. A cytoplasmic fiber detached from an array of chloroplasts travelled in the medium at a speed of $\sim 20 \mu\text{m/s}$. Arrows show the tip of the fiber. Bars, $10 \mu\text{m}$. $\times 1,200$.

FIGURE 9 The conversion of a travelling fiber into a rotating ring. Successive photographs were taken at time intervals of $\frac{1}{2}$ s. A travelling fiber was converted into a rotating ring after travelling for a long distance. The short arrow in *a* indicates the tip of the fiber and the long arrow in *a* indicates the tail of the fiber. In *c*, the fiber began to round up at the position indicated by the short arrow. Bars, $5 \mu\text{m}$. $\times 1,550$.

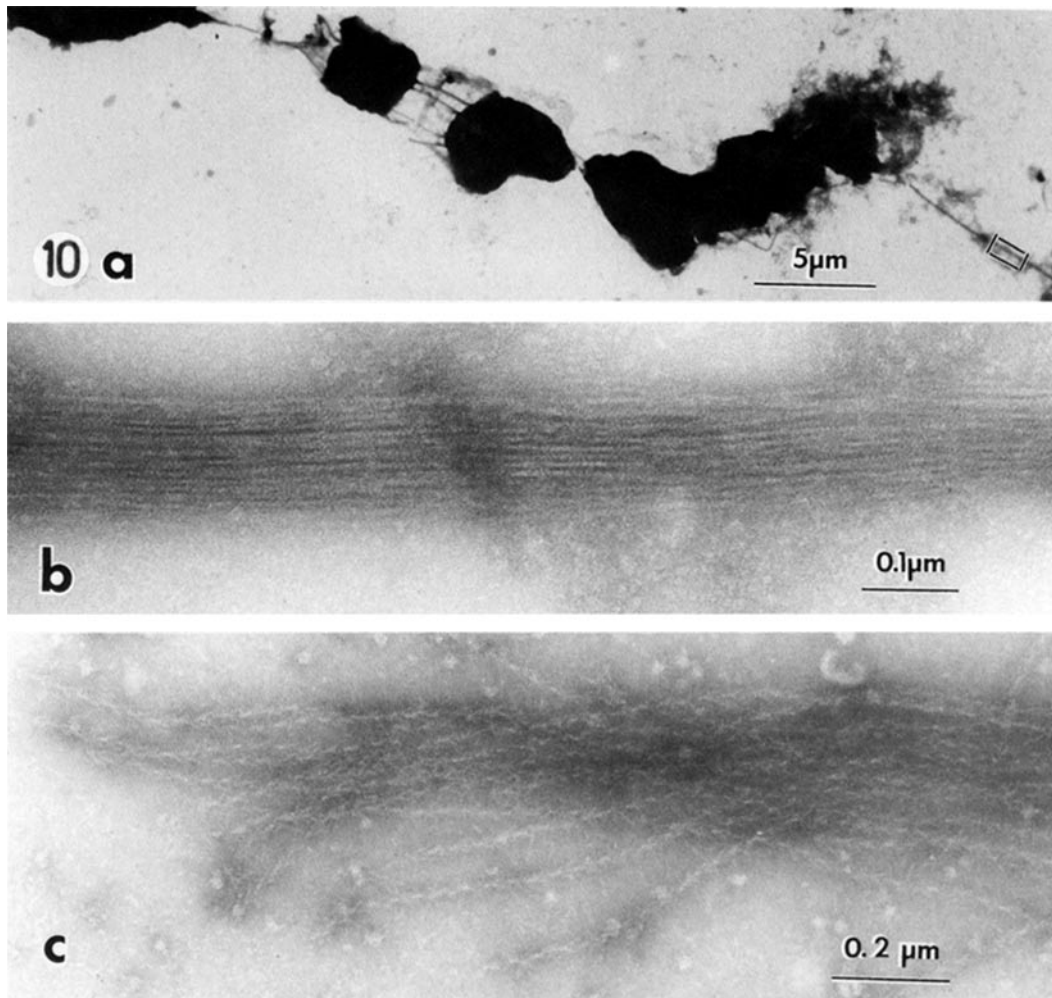


FIGURE 10 Electron micrographs of the cytoplasmic fiber connecting a chloroplast chain. (a) A chloroplast chain isolated in the activating medium was viewed at low magnification. $\times 3,200$. (b) When the area enclosed by a square in *a* was observed at a higher magnification, the fiber was found to be composed of a bundle of thin filaments. $\times 127,000$. (c) These thin filaments were capable of binding with muscle HMM. All arrowheads of acto-HMM point in the same direction. $\times 77,000$.

a translational motion of the filaments. This idea, however, is not acceptable, because after repeated perfusion with the fresh medium, there could have been only a few free myosin molecules in the medium.

The above idea could be modified in the following way: Myosin molecules interacting with F-actin would not detach from F-actin, but walk on an F-actin by alternative binding of two heads. This mechanism would explain the rotation of rings caused by continuous walking of myosin, but the travelling of fibers could not be explained because myosin molecules must finally dissociate from the ends of F-actin filaments after walking along the fiber. Another possibility might be that myosin molecules, after the active stroke on an F-actin, recover the original conformation for the next effective stroke, just like cilia. However, the electron micrographs of the cytoplasmic fibers isolated in the medium did not give any indication that myosin molecules kept binding to the fibers.

The following idea should be considered also: The movement of the cytoplasmic fibers could be produced as a result of their interaction with some proteins on the surface of the glass slide. Actually, after perfusion of the medium, the moving fibers were observed only very close to the glass surface, within the depth of focus of the microscope, of $\sim 1 \mu\text{m}$. If protein molecules

were attached to the glass surface, they could not be easily washed away. If the tails of myosin were bound to the glass surface and the heads could interact with F-actin, the bundles of F-actin filaments would continue moving on the surface. We do not know whether or not myosin molecules were bound to the glass surface in a sufficient number to move the fibers, or whether the moving fibers were so close to the surface that cross-bridges could form between the fibers and myosin molecules on the surface.

At present, I have no conclusive idea on the mechanism of the travelling of fibers and the rotation of rings.

Sucrose and Mg-ATP were indispensable for inducing the movement of fibers. Mg-ATP must supply the energy for the movement. The role of sucrose is unknown. In the case of muscle contraction, Ca^{2+} activates the actomyosin ATPase and induces contraction. On the other hand, the removal of Ca^{2+} facilitated the movement of fibers. This is consistent with the previous reports that the concentration of Ca^{2+} must be lower than 10^{-7}M for the movement of granules along the subcortical fibrils (6, 32).

The cytoplasmic fibers from *Nitella* were composed of bundles of F-actin filaments. Therefore, the effect of muscle proteins, actin, myosin, or HMM, and tropomyosin was examined.

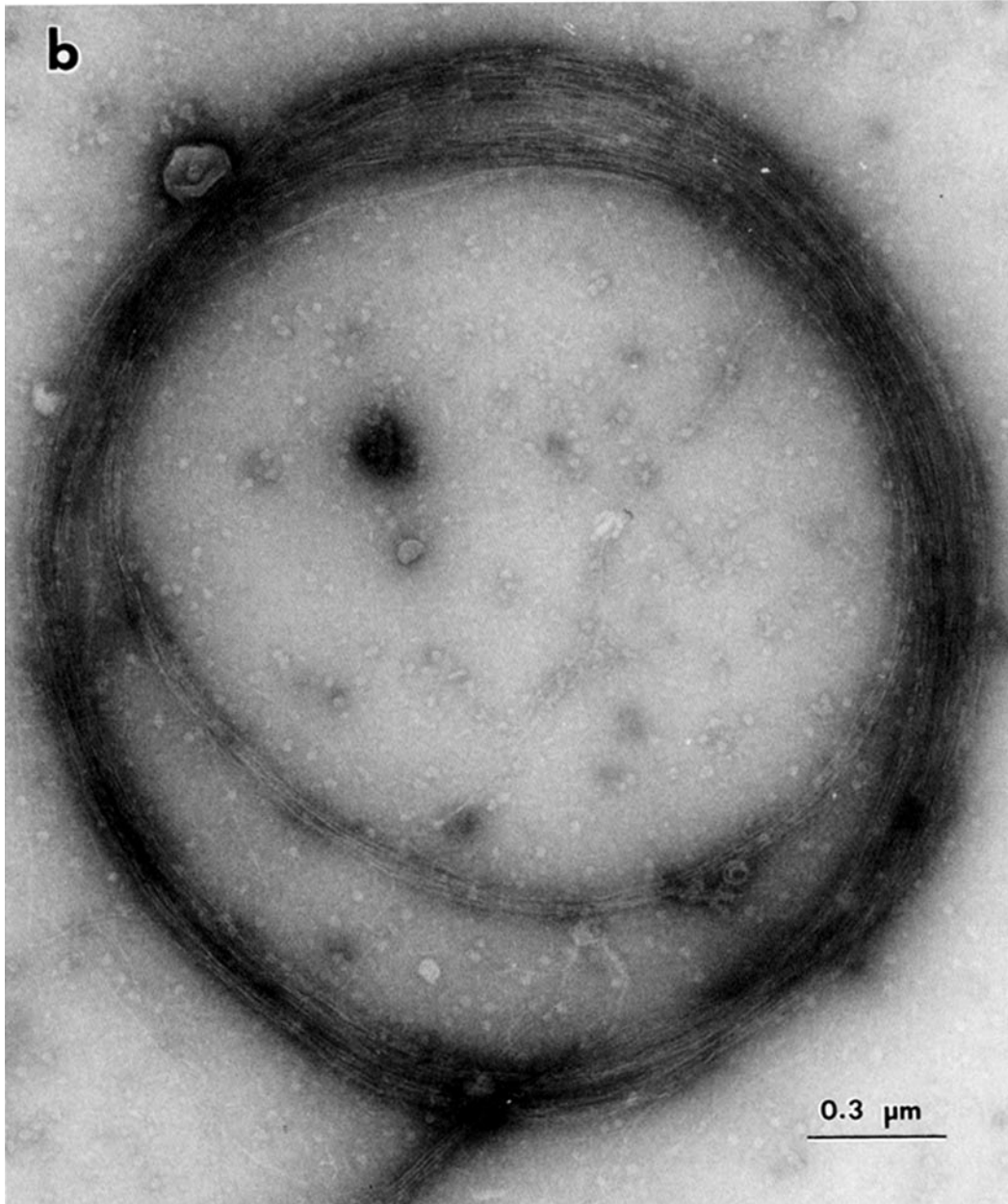
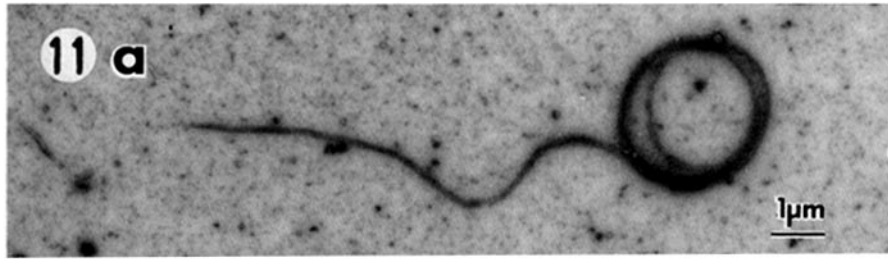


FIGURE 11 Electron micrographs of the rotating ring. (a) A ring with an unrounded tail of the fiber is shown at a low magnification. This fiber seems to be under conversion from the travelling fiber to the rotating ring. $\times 6,600$. (b) Enlarged photograph of an area of the ring in a. The ring consists of three to four turns of a bundle of thin filaments. $\times 62,400$.

At concentrations of 0.1–0.3 mg/ml of each protein used, none had an appreciable effect on the movement of the fibers.

There were two distinct states of the cytoplasmic fibers: motile and nonmotile. In the nonmotile state the fibers or the chloroplast chains lost their tension. It was previously found by Kamitsubo that slack fibers did not participate in endoplas-

mic streaming but fluttered passively in the stream (13). As described in Results, motile and nonmotile parts coexisted even in a single chloroplast chain. There may be a third protein that can regulate the state of F-actin filaments, although there is no report of actin-binding proteins in *Nitella*.

The molecular mechanism of movement, the travelling and

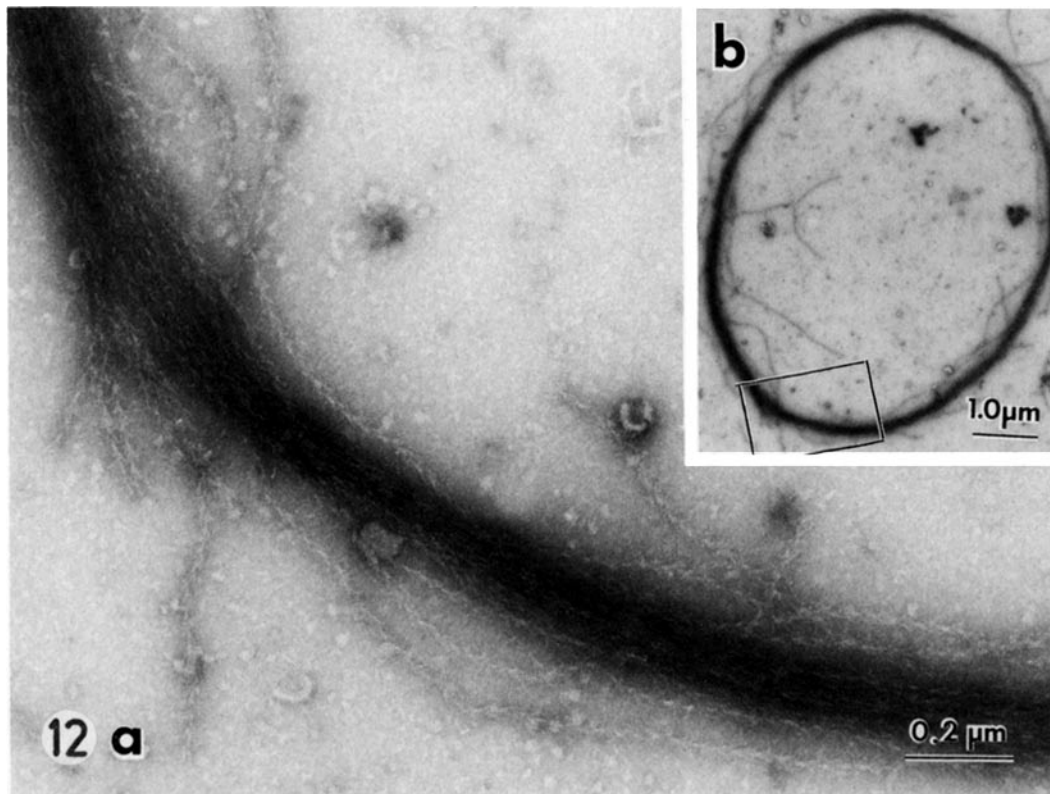


FIGURE 12 Rotating ring decorated with muscle HMM. (a) All arrowheads point in the same direction. Clear figures of arrowheads are visible on peripheral fibers of the bundle. The area enclosed with a rectangle in *b* is observed at a higher magnification. $\times 70,400$. (b) Entire view of the ring decorated with HMM. $\times 8,300$.

the rotation, of the cytoplasmic fibers remains mysterious. However, I believe further investigations along the line of this work will be useful to construct various models of cell motility and eventually elucidate the mechanism of motility.

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