MOVEMENT GENERATED BY INTERACTIONS BETWEEN THE DENSE MATERIAL AT THE ENDS OF MICROTUBULES AND NON-ACTIN-CONTAINING MICROFILAMENTS IN STICHOLONCHE ZANCLEA

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ABSTRACT

Axopods of the planktonic protozoan, Sticholonche, are used as oars to propel the organism through seawater. Within each axopod is an organized array of microtubules which inserts into a dense material that assumes the form of the head of a hip joint. This material, in turn, articulates on the surface of the nucleus. Microfilaments, 20–30 Å in diameter, connect the dense material associated with the microtubules to the surface of the nucleus, and they move the axopod by their contractions. The active phase of the movement may take as little as about 0.04 s, and the recovery phase may take between 0.2 and 0.4 s. The microfilaments are not actin, as based on: (a) their small diameter, (b) the lack of decoration with heavy meromyosin, and (c) their ability to coil, spiral or fold during contraction. By the use of Thorotrast, we were able to demonstrate that the cell surface is deeply infolded, extending all the way to the hip joint. Here, there is a specialized membrane system that resembles the diad in skeletal muscle. From cytochemical tests and the use of ionophores and chelators, there is some evidence that the motile process may be controlled by calcium. This study demonstrates that, in at least one system, microtubules can be moved by contractile microfilaments attached to the dense material at their tips.

Three basic mechanisms, which are dependent upon the integrity of microtubules, have been proposed for cytoplasmic movements such as chromosomal movements during mitosis and meiosis. In one scheme, originally postulated by Inoué and his co-workers (22, 23), the chromosomes are moved by the controlled assembly and disassembly of microtubules. A second scheme visualizes chromosomes being moved by the sliding of microtubules past one another by dynein-like bridges (28). More recently, a third postulate has come into vogue in which actin filaments and microtubules interact in some undefined way to provide movement (11). There is some evidence for each scheme; assembly and disassembly must occur as the chromosomal distance decreases, there are ATPases in the spindle (33), and there is some morphological evidence for the presence of bridges (27), and finally actin is present in the spindle as defined by heavy meromyosin (HMM).
binding (13, 11, 37). Although many experiments have been performed and much speculation has been published, there is so far no easy way to distinguish between these alternatives or in fact to demonstrate that one or more of the above possibilities, perhaps all three, act to effect chromosomal movement or, for that matter, other microtubule-associated motile events such as secretion, streaming, etc.

In this paper, we present a motile system in which microtubules are moved by microfilaments in a novel way. The system that we describe is present in the protozoan, *Sticholonche zanclea.* This extraordinary planktonic protist contains axopods which it uses like oars to row itself through the ocean depths. Within each axopod is an organized bundle of microtubules which inserts into a plaque composed of a dense material (18). Microfilaments, in turn, connect to this dense plaque and from there insert on the nuclear capsule. We set out to describe the motion of this organism and the nature of its filaments. In the course of this study, we discovered that the filaments are not actin and that they shorten presumably by supercoiling. Yet these filaments produce motion in a manner unlike that for motion described elsewhere; the microtubules are moved by being pulled by filaments attached to the dense material at their ends. Filaments similar to the microfilaments in this system may exist in other cells but, because of their slender dimensions and their tendency to fold or coil, they would be virtually indistinguishable from the cytoplasmic "ground substance." Yet, the motion of particles in cells may be effected by microtubules being pulled through the cytoplasm by a contractile apparatus attached to one of their ends. It is the purpose of this paper, then, not only to describe an extraordinary biological system, but also to suggest that the current postulates about the mechanism of microtubule-associated motility are defined too narrowly. Even chromosomal movement may be affected in ways not yet reckoned with.

**MATERIALS AND METHODS**

**Obtaining Material**

*Sticholonche zanclea* Hertwig were collected in plankton samples taken at depths of 100-300 m near Villefranche-sur-Mer. They were isolated from other planktonic organisms by pipetting them individually from the plankton samples; this was carried out under a dissecting microscope. Once isolated, they were maintained at 14°C in seawater collected far from the coast since these protozoa are sensitive to the pollution in the seawater near Villefranche. Shortly after they had been isolated and placed in fresh seawater, they began to move actively.

**Light Microscope Observations**

The motion of *Sticholonche* can be most conveniently observed with a dissecting microscope at magnifications of × 50-100. For detailed observation of the axopods, *Sticholonche* were examined with a Leitz compound microscope. Movies were taken with a 16-mm Beaulieu camera coupled to the Leitz microscope at 25-35 frames.

**Experimentation on Living Sticholonche**

**Removal of the Axopods**

By sucking *Sticholonche* up and down through a pipette whose diameter was just a little larger than that of the nuclear capsule, it was possible to remove the bulk of the cytoplasm, leaving only the bases of the axopods and the capsule. The amount of denudation of the cells was monitored by the dissecting microscope, and the pipetting was repeated if necessary. The motion of the axopod bases could then be analyzed.

**Application of Seawater Containing Low Calcium**

*Calcium-free seawater:* The artificial seawater used was equal in salinity to the seawater in the Mediterranean (39.6 ppt). It contained 516 mM NaCl, 10.8 mM KCl, 34.0 mM MgCl₂, and 6.0 mM Na₂SO₄. Calcium-free seawater made up as indicated above actually contains considerable calcium due to contamination of the chemicals with calcium, the greatest quantity of calcium coming from the MgCl₂. To the artificial calcium-free seawater, we added sodium oxalate in some experiments; in others we added a chelator of calcium, namely ethylene glycol-bis(β-aminoethyl ether)N,N',N''-tetraacetate (EGTA). A 0.1 M stock of EDTA was made up and neutralized with NaOH. In all cases, the pH of the seawater was checked and adjusted, if necessary, before use.

**Use of SODIUM OXALATE IN NATURAL SEA-WATER:** Natural seawater was also deprived of calcium by the addition of 1% sodium oxalate (~0.075 M). After 2 days the seawater was centrifuged to remove the calcium oxalate precipitate.

**Action of Ionophores**

The ionophore X537A (courtesy of Hoffmann-La Roche, Inc., Basel, Switzerland), was dissolved in 1 vol of dimethyl sulfoxide and 3 vol of ethanol. A stock of 1 mg/ml of the ionophore was made up. 10-15 μl of this stock were added to each milliliter of seawater containing *Sticholonche.*

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Scanning Microscope

Organisms freed of much of their cytoplasm by forcing them through a narrow-bore pipette were fixed in 1.25% glutaraldehyde and 2% paraformaldehyde in seawater for 1 h, washed in seawater and in distilled water, and deep frozen in nitrogen at its melting point (−210°C). The organisms were then freeze-dried at −90°C in Leybold-Hae rueus EPA 100 apparatus coated with gold-palladium alloy and observed with a Cameca MEB O 7 scanning electron microscope.

Transmission Electron Microscope Techniques

Untreated Sticholonche or Sticholonche after experimental treatment were fixed by a method modified from that of Karnovsky (25). The protozoa were fixed in 1.25% glutaraldehyde and 2% paraformaldehyde in 0.4 M phosphate buffer at pH 7.2, for 30 min to 1 h at room temperature, washed in 0.4 M buffer, and postfixed in 2% OsO4 in 0.4 M phosphate buffer at room temperature. Some specimens were dehydrated immediately; others were stained en bloc after washing in 0.5% uranyl acetate or uranyl acetate containing CaCl2 (26) for a few hours to overnight, dehydrated in ethanol, and embedded in the low viscosity medium of Spurr (40). Thin sections were cut with a diamond knife on a Porter-Blum II ultramicrotome, stained with uranyl acetate and lead citrate, and examined in an Hitachi HU 11 A electron microscope at the Laboratoire de Protistologie Marine of the University of Nice.

Mordant Staining with Tannic Acid

To better visualize the substructure of the membranes of Sticholonche after pre- and postfixation, Sticholonche were washed in 0.05 M phosphate buffer and then stained with 1% tannic acid (most of the tannic acid contained digallic acid which was obtained from Mallinkrodt Inc., St. Louis, Mo.) in 0.05 M phosphate buffer at pH 7.0 overnight. The Sticholonche were then washed in 0.1 M phosphate buffer for 5 min, dehydrated, and embedded as above. This procedure was slightly modified from that described by Simionescu and Simionescu (38).

Determination of Extracellular Space by Use of Thorotrasst

We employed the procedure of Rambourg and Leblond (35). After pre- and postfixation, Sticholonche were washed in 3% acetic acid (pH 2.6), incubated for 24 h in a 1% solution of colloidal thorium (obtained from Taab Laboratories, Emmer Green, Reading, England), dissolved in 3% acetic acid, washed in 3% acetic acid, and dehydrated and embedded as above.

Localization of Calcium

For the localization of calcium, we employed the method of Carasso and Favard (5). Sticholonche were fixed in 2% glutaraldehyde buffered with 0.6 M phosphate at pH 7.8-8.2 for 45 min. They were washed three times in water to which NaOH had been added to raise the pH to 8.0, then incubated in a 5% solution of lead acetate for 20 min, washed in Veronal acetate buffer and postfixed in 2% OsO4 in Veronal acetate buffer (pH 7.2) for 40 min, dehydrated, and embedded.

Treatment of Cells with Detergent Sticholonche were treated with 1% Triton X-100 (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, New York) in 50 mM Tris HCl, 3 mM MgCl2, and 0.1 mM EDTA (ethylenediaminetetraacetate) at pH 8.0 (solution referred to as TTM). Cells were then fixed and processed as outlined above.

Heavy Meromyosin Decoration

Heavy meromyosin (HMM) was prepared from rabbit skeletal muscle and stored in 50% glycerol at −20°C. The HMM was kindly supplied by Dr. J. Sanger and Dr. A. M. Weber of the University of Pennsylvania. Each batch was assayed for its activity by the addition of HMM to purified rabbit actin prepared from an acetone powder of skeletal muscle. The assay was made by negative staining and by thin sectioning.

Sticholonche were treated with TTM for 3-4 min, then placed into a dialysis sac containing HMM (6 mg/ml in 50% glycerol) and dialyzed overnight against a large volume (500 vol) of 60 mM KCl and 30 mM Tris at pH 7.3. In one experiment, trout myofibrils were included in the same dialysis sac to insure that the HMM was binding normally; in another experiment, TTM-treated copepods were placed in another dialysis sac containing HMM. All operations were carried out at 0°C. The protozoa were collected, washed thoroughly in buffer solution to remove any unbound HMM, and fixed as outlined above. After osmium tetroxide postfixation the cells were washed in distilled water and stained in 0.5% uranyl acetate overnight. They were then dehydrated and embedded.

RESULTS

As described by Hollande and Enjumet (19) and Hollande et al. (18), Sticholonche zanclea is a marine planktonic organism probably belonging to the order Heliozoa (4). The size varies, the mean length of the cell body being about 200 μm. Extending from the cell body are 14 rosettes of siliceous spicules and numerous locomotory axopods. The former insert only in the superficial cortical cytoplasm; the latter insert in rows of little cups formed on the surface of the large (≈80 × 25 μm) centrally located nucleus (Fig. 6). Sticholonche are bilaterally symmetrical with a convex dorsal surface, and their anterior end is swollen. This symmetry is reflected in the morphology of...
the nuclear surface (the nuclear capsule) which is convex on its dorsal surface, yet concave on its ventral surface. The axopods, which average 150 \( \mu \text{m} \) in length, are straight, rigid, and transparent (Fig. 1). The organism moves by using these axopods like oars such as one might envision a Greek galley (trireme) progressing through the water, only in this case it is submerged. The axopods are arranged into two basic groups, a dorsal group and a ventrolateral group (Fig. 2). The dorsal group is divided into two halves by a central dorsal midline space. Each half is composed of about 160 axopods arranged into three rows which run from the anterior end of the organism to its posterior end. The dorsal axopods do not move. Clearly separated from the dorsal axopods by a space which takes the form of a sulcus (see Fig. 1 and the large arrows in Fig. 2) are the dorsolateral, lateral, and ventral axopods, all of which are motile. These axopods are also organized into rows which run from the anterior end of the organism to its posterior end. The anterior axopods are larger than the

Figure 1 Single frames from a movie sequence of the motion of the axopods of Sticholonche. The film was taken at 30 frames/s, and every fourth frame was printed. The arrows indicate the movement of the dorsolateral axopods. The letter D indicates the nonmotile dorsal axopods. \( \times \) 300.

Figure 2 Drawing of a section through Sticholonche. The orientation of the organism is similar to that in Fig. 1. The arrows indicate the relative movements of the four types of axopods, the ventral (V) lateral (lat), dorsolateral (D. lat), and dorsal (D). The dotted arrows indicate the recovery stroke of the axopods. The large arrows adjacent to the dorsal axopods show how the sulcus is eliminated.
posterior ones. In the posterioventral end of the organism there are no axopods.

**Motility**

It is important to establish at the outset that it is the axopods which move, effecting the movement of the spines and thus the organism. The movement of the axopods not only results in a forward translation of the organism through the water but also appears to keep the animal from settling to the bottom. Thus, when the organisms cease to move, they fall to the bottom of the dish. The movement of the axopods can be demonstrated by carefully observing movies of organisms; in the movies the movement of the spines always follows the movement of the axopods. We have also observed that the axopods in *Sticholonche* that are spineless display normal rowing movement. Spineless *Sticholonche* occur naturally; alternatively, the spines can be removed along with the distal three-fourths of the axopods and some of the cytoplasm by sucking *Sticholonche* through a narrow-bore pipette (Figs. 3 and 4).

After this treatment the bases of the ventrolateral axopods move normally in many of the organisms. Thus, although the spines move, their movement appears to be dependent upon the active movement of the axopods.

The translational motion of the organisms can be easily documented. During the active stroke the organism moves forward one unit. There is a transitory pause and, then, as the axopods return in their recovery stroke the organism retreats about two-thirds to three-fourths of a unit. Again, there is a transitory pause and the active stroke begins again. The resultant movement is forward but rather inefficient as the forward motion is balanced by a partial retreat during the recovery stroke. The axopods accomplish this motion by a metachronal rhythm beginning at the anterior end of the organism.

The number of strokes per second at a constant temperature varies from organism to organism and depends to some extent upon the health of the organism. For example, just before an organism dies, the number of strokes

![Figure 3](https://example.com/figure3.png)

**Figure 3** Scanning electron micrographs of a *Sticholonche* which has been denuded of much of its cytoplasm by being sucked through a narrow-bore pipette. The bases of many of the axopods remain attached to the nuclear capsule. The anterior end of the cell is to the right, as can be determined by the larger axopods. × 1,250.
The organism was denuded of much of its cytoplasm as in Fig. 3. The 10 pictures illustrated here are alternate frames from a movie sequence. This sequence shows one cycle of the movement. Of particular interest are frames h and i. The arrows indicate the movement of the ventral axopods. × 600.
per unit of time increases markedly. Healthy organisms perform one to three strokes per second. In each organism, however, at any one moment the rate of movement is constant; in fact, the movement resembles the rhythmic pulsations of a heart.

As already mentioned, the dorsal axopods do not move or move to no appreciable extent. The ventrolateral axopods show different modes of movement depending upon the position, lateral or ventral. Maximally, they can move through 120° of arc in less than 0.1 s. We begin with a description of the ventral axopods.

An analysis of movies of an organism from which the spines and the distal ends of the axopods have been removed by forcing it through a small pipette reveals the following (sequences are shown in Fig. 4). For this organism, there are about 1.5 strokes/s. The ventral axopods have an active stroke that progresses anteroposteriorly; in the recovery phase the axopods return using the same path. For these axopods, the active stroke is completed in one frame of the movie (Fig. 4) showing that the minimum speed of motion of this axopod is 0.04 s. (It should be remembered that the speed of an axopod from an untreated organism is slower than this [see Fig. 1].) This active phase is followed by a complete cessation of motion for 0.2 s. The recovery phase begins and lasts 0.23 s, and after another pause of 0.2 s, the cycle is repeated. The important points are that the recovery phase is six times slower than the active phase and that the active phase is immediately preceded and followed by a phase of no motion at all.

The dorsolateral axopods, on the other hand, instead of showing a cycle in which the axopods go and return on the same path, follow a triangular, nearly circular route (Fig. 2). For these axopods there is short active phase in which the axopods move posteriorly; this is followed by a brief period during which there is a cessation of movement. During the recovery stroke the axopods first move downwards followed by an upward movement. Unlike the ventral axopods, at the end of the recovery stroke, the axopods immediately begin the active stroke with no phase during which motion is arrested. In the recovery phase the tip of the axopod follows the course of half of an ellipse (see Fig. 2). The time for the recovery stroke is very long relative to the active stroke. Exact times were not measured in this case because the axopods continually moved in and out of the focal plane.

The lateral axopods trace an elliptical path which is intermediate in shape between the triangular path of the dorsolateral axopods and the linear (rectangular) path of the ventral axopods. Movies of untreated organisms demonstrate activities similar to those described for the denuded organisms, although in most cases the rowing speed is faster in denuded organisms.

To decide which group of axopods gives rise to most of the forward translation and to the upward movement is beyond the scope of this report, especially when one considers that the spines must contribute to the net movement as must the progressive elimination of the sulcus (this region is indicated by the larger arrow in Fig. 2) which lies between the dorsal and dorsolateral axopods. As the metachronal wave passes posteriorly, this region is lost. Nevertheless, there are several points which are clear and should be emphasized. First, the active phase is considerably shorter than the recovery phase. Secondly, there appear to be phases or a phase during which no motion is going on, and, finally, the axopods move in a metachronal fashion which leads to a progressive loss in the width of the sulcus. The movements described here were shown in a movie presented to 15th Reunion du Groupement des Protistologues de langue française in Roscoff, France (3).

Ultrastructure of Regions Responsible for Motility

The basic morphology of Sticholonche has been described in an earlier report (18); therefore, we concentrate on only those morphological details which are relevant to understanding how the motion is generated. In subsequent reports we will describe in detail many of the morphological features such as the arrangement of extracellular layers, the relationship of the microtubules in the axonemes with the plasma membrane and the extracellular fibrils, details of the nuclear capsule, the form and formation of the spines, etc.

Each axopod is composed of an organized bundle of microtubules which inserts into a dense material; the overall form of this dense material resembles the head of a hip joint (Fig. 5). The socket into which the head of the hip
joint articulates is a cuplike depression formed in the surface of the nucleus (Figs. 5 and 6). Let us begin with a description of the nucleus.

**Nuclear Capsule:** The surface of the kidney-shaped nucleus is studded with shallow depressions (sockets) organized into rows which run the length of the nucleus (Fig. 6). The largest sockets are located on the anterior end of the nuclear capsule, corresponding to the position of the largest axopods. There are no axopods on the posteroventral portion of the organism where the nuclear capsule is very thin and lacks sockets. Thus, the precise ordering of axopods into linear rows is brought about by the organization of the surface of the nucleus.

The surface of the nuclear capsule is extremely stable. It is possible, for example, to force *Sticholonche* through very small-bore pipettes, a procedure that will remove all the cytoplasm which includes the heads of all the hip joints. The surface is unchanged after this process.

**Figure 5** Thin section through a portion of the nucleus (N) and the axonemes (A) which articulate on the surface of the nucleus. Of particular interest is the fact that the axonemal microtubules insert into a dense material which in turn takes the form of the head of a hip joint. Filamentous bundles (see arrows) connect this dense material, that makes up the head of the hip joint, with its socket, the nuclear capsule (nc). × 20,000.

**Figure 6** Phase-contrast micrograph of an isolated nucleus of *Sticholonche*. The surface of the nucleus (nuclear capsule) contains linear rows of cuplike depressions. These depressions are the sockets for the axonemes. The anterior end of the nucleus with its larger axopods is located on the left of the micrograph. × 400.
treatment; in fact, it is difficult to rupture the nucleus. Furthermore, this surface resists extraction with detergents such as Triton X-100 (see below). We will describe the organization of the nuclear capsule in a subsequent report, but suffice it to say, the nucleus is limited by a normal nuclear envelope. The rigidity of the nuclear capsule seems to be brought about by the organization of material which lies directly beneath this envelope (within the nucleus).

**Axopods and Axonemes:** Within each axopod is an axoneme composed of a highly ordered array of microtubules (Fig. 7). The shape of the axoneme in transverse section depends upon the species of axopod one is studying and can vary from nearly round to rectangular. Most often the axoneme takes the form of a hexagon with unequal sides. Each microtubule, 220 Å in diameter, in the axoneme is linked to three others (Fig. 7), forming a packing which looks like numerous linked hexagons. One hexagon composed of six microtubules extends farther than the others into the dense material at the head of the hip joint (Figs. 5, 11, and 12).

In a longitudinal section (Fig. 8), adjacent microtubules are seen to be joined by prominent bridges. These bridges can be identified irrespective of whether they lie within the plane of section or whether they project towards the viewer and thus are cut perpendicular to their axes. In the latter case they appear as small densities. The bridges are periodic with a repeat of 320 Å.

**Detailed Description of the Point of Articulation Between the Axoneme and the Nuclear Capsule:** Since we know that the basal end of each axopod moves as a unit (i.e. in denuded organisms, Fig. 4), with different paths depending upon its position on the animal, ventro- or dorsolateral, we now focus on this region, for it is here that the motion must be generated.

We have already demonstrated (Fig. 5) that the microtubules in each axoneme insert into a dense material which in its simplest form takes

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**Figure 7** Transverse section of an axoneme of Sticholonche. This axoneme is composed of microtubules organized such that each microtubule is bridged to three others. × 112,000.
FIGURE 8 Longitudinal section of the axoneme of Sticholone. Of particular interest are the bridges which are periodic. The small dense dots indicated by the arrows are bridges cut in transverse section. × 132,000.

...the shape of the head of the femur of a hip joint. This material in turn inserts into a socket in the nuclear capsule. The three different types of axopods, i.e. dorsal, ventral, and lateral, have variations in the position of the "head of the femur" relative to the axoneme, in the morphology of this unit and in the distribution of microfilaments in the socket. These variations appear responsible for the unique motion or lack of motion of the different types of axopods.

For example, the lateral axopods have an articulation which closely resembles a hip joint with a prominent hemispheric ball extending from the plate on which the microtubules insert (Fig. 9). This allows movements of large amplitude which could occur in all planes. In contrast, the dorsal axopods which do not move have an articulation that resembles that of adjacent vertebrae. The ball or balls attached to the plate on which the axoneme inserts are very thin or flat; thus movement would be minimal. And finally, the ventral axopods have an elongated, almost rectangular, shape in which the ball is positioned towards one end of the rectangle. This would allow movement in only one plane, forwards and backwards.

The axopodial complex can occupy three basic positions relative to the nuclear capsule: it can exist perpendicular to a tangent drawn to the nuclear capsule at the point of articulation (Fig. 9), and it can be inclined relative to this tangent such that the axopod points either towards the anterior end of the organism or towards its posterior end (Fig. 10). The maximum angle of inclination varies depending upon the axopod, but it can be greater than 45° either side of a perpendicular drawn to the socket. In fact, it may be as much as 60° on either side. In all positions the microtubules remain bridged to each other in the same way and, perhaps more importantly, their relationship with the dense material making up the head of the femur is unaltered. Thus, during movement, the microtubules making up the axoneme appear to move with the dense material as a unit, just as the femur moves in its socket. There appear to be no alterations in the connections between the microtubules and the dense material such as gross bending or distortion of the dense material. Since this dense material is not changed, the motion does not seem to be effected by any type of sliding interactions between the microtubules. Instead, since the length of the microfilaments changes markedly (Fig. 10), the movement seems to result from interactions between the microfilaments which extend from the lateral surfaces of the dense plaque to their insertion points in more dense material attached to the nuclear envelope.

THE MICROFILAMENTS: Let us begin a description of the microfilaments by examining the stage at which the axopodial complex rests perpendicular to the socket, not inclined at an angle to it. In thin sections through the hip joint at this stage, we see a set of microfila-
Fm~ 9 Section through the base of an axoneme. The dense head of the hip joint articulates on the nuclear capsule (nc). Of particular interest are the microfilaments on either side of the head of the hip joint. × 50,000.

Figure 9. Section through the base of an axoneme. The dense head of the hip joint articulates on the nuclear capsule (nc). Of particular interest are the microfilaments on either side of the head of the hip joint. × 50,000.

ments either on both sides of the head of the femur (Fig. 9) or only one side indicating that the microfilaments are not attached around the entire margin of the dense plaque. We know now that for the ventral axopods there are only two groups of filaments on opposite sides of the hip joint. One of these groups is larger than the other, and this group is in a position to account for the active stroke by contraction. The smaller bundle is in the requisite position for the recovery stroke (a detailed description will be presented in a subsequent report). The filaments extend from the edge of the plaque to a dense material situated on the nuclear envelope. The diameter of the filaments is 20–30 Å as can be measured in longitudinal (Figs. 9, 13) or transverse section (Fig. 11), although we often see thicker filaments. Careful examination of the micrographs suggests that these thicker filaments may result from a twisting together of several 20–30 Å filaments (see arrows in Fig. 14) but, because the section thickness is at least 10 times greater than the diameter of an individual filament, we cannot rule out the possibility of superposition.
FIGURE 10 Section through the head of the hip joint when it is inclined to one side. Of particular interest is a comparison of the filaments (f) on the left of the hip joint with those on the right. On the right, the filaments are difficult to distinguish. Instead, one sees a series of bands (see arrows). × 50,000.

FIGURE 11 Transverse section through the base of a hip joint. In the center of the dense material is a circlet of six microtubules. Around the dense material are transverse and slightly oblique sections of the filaments. In transverse section, individual filaments appear as small dense dots. Clusters of dots appear as thicker units. The arrows indicate that the edge of the hip joint seems to be made up of dense bars. × 75,000.
artifacts. If one examines the bundle of filaments as a unit, it often appears as if the filaments were organized into a series of bands—dense and less dense bands (Figs. 10 and 13). It is tempting to interpret these dense bands as areas where the filaments coil or twist around each other, and the less dense bands as areas where the filaments run linearly (see the drawing associated with Fig. 13).

The microtubules in the axonemes insert into a dense material which consists of a series, generally four to five, of flat plaques. Basal to these plaques is some more dense material some of which forms the head of the femur. The microfilaments appear to insert into this dense material and appear to be continuous with it. A similar dense material is present on the point of insertion of the microtubules with the socket (Figs. 9, 10, and 12). In both cases the dense material has a granular texture. At higher resolution this material appears to be made up of linear rods which, on careful examination, are composed of material organized into the form of a spiral (Figs. 11 and 12). Frequently, the microfilaments appear to be continuous with these spiral rods. Thus, it seems that the microfilaments in the dense regions may coil about themselves and each other as well as supercoil (see the legend for Fig. 13).

When the axopod is inclined at an acute angle, the filaments on either side of the hip joint are clearly of different lengths and show different morphologies. On the side on which they are the shortest, the bundle of filaments tends to be broader and often one sees a series of dense bands (nine is the maximum number of bands observed). These bands are often not visible when the axopodial complex is inclined maximally (Fig. 10). On the side opposite the acute angle of inclination, the filaments are very long and tend to be separated from one another so that it becomes relatively easy to

![Figure 12](image-url)

**Figure 12** Section similar to that illustrated in Fig. 11. The arrows indicate that the bars making up the dense material appear to be composed of material organized into a tight spiral. Microfilaments (f) are present on either side of the hip joint. x 92,500.
measure their dimensions. When maximally elongated, the filament clusters rarely show alternating dense bands, as opposed to the situation where the axopodial complex makes an acute angle with the socket as when the filaments have shortened. In many cases, it appears that the amount of dense material attached to the nuclear envelope is reduced in the region where the filaments are more extended. The differences in the lengths of the filaments attached to the two margins of the same dense plaque when the axopodial complex is inclined maximally are extraordinary. At maximum extension the filaments measure 1.3 μm, whereas

Figure 13  (a) A microfilament bundle from an axopod which was partially contracted. The arrows indicate dense bands. Between the arrows the microfilaments can be seen as linear elements. Our interpretation of the material in the dense bands is illustrated in the tracing of this micrograph (b). × 115,500.
the shortest length is only 0.13 μm; thus, these filaments can shorten 9–10 times their extended length.

In some organisms we found that the filaments, instead of appearing as single units, tended to form bundles as if there might be some lateral aggregation. When we treated Sticholonche with the detergent, Triton X-100 in buffer (TTM), for example, this tendency to aggregate became marked (Fig. 14). Sometimes, these clusters of filaments would appear striated as well (Fig. 14). This lateral association of the filaments can be documented in transverse sections where, instead of seeing small dense dots, we see large dense masses which cannot be resolved into individual filaments, indicating that the individual filaments have twisted around each other. It appeared that the medium in which the cell was placed before the addition of detergent made a difference; for example, if the organism was in calcium-free seawater first, the filaments tended to aggregate less when the organism was placed into the detergent. Likewise, the addition of EGTA tended to inhibit the lateral association of filaments.

**THE NATURE OF FILAMENTS:** Although the microfilaments here are too small to be actin and their behavior (i.e. they appear to shorten by coiling or folding) is not actin-like, we wanted to see whether these filaments would bind HMM. After demembranation with the detergent Triton X-100, the organism was placed in HMM. No decoration of the microfilaments was seen (Fig. 15), even though the experiment was repeated. The actin filaments in trout myofibrils which, in one experiment, were added to the detergent-treated Sticholonche did decorate, however, as did the actin filaments in copepod muscle, showing that the HMM was active and would bind under the conditions employed during this experimental procedure.

**Control of Axopod Motility**

We have already stated that the axopods beat with a metachronal rhythm indicating a high degree of coordination between axopods. One wonders how this metachrony is achieved and how the cell performs an active stroke followed by a pause and then a recovery stroke. The observations and experiments presented below address themselves

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**Figure 14** Section through the base of an axoneme from an animal which has been extracted by the detergent Triton X-100. Note that the microfilaments aggregate under these conditions to form bundles. One of the filament bundles indicated by the arrow is striated. The filaments in the bundles tend to twist around one another. × 132,000.
to a preliminary definition of such a control mechanism.

**The Relationship of the Plasmalemma to the Axoneme:** From the morphology of the region between the dorsal axopods and the lateroventral axopods, and from observation of the motile behavior of organisms which have been denuded of much of their cytoplasm, we suspected that the plasmalemma was deeply infolded. Hollande et al. (18) demonstrated a population of filaments which run between adjacent axonemes and which, from their preliminary observations, appeared to be contained within a membrane compartment separated from the microtubules. To establish whether indeed these filaments are extracellular and, thus, unlikely candidates for effecting the motive force, and to determine exactly how far the external environment penetrates into the cytoplasm of *Sticholonche* (a subject which would be of interest in any discussion of the control of motility), we fixed organisms in the presence of Thorotrast (Fig. 16). This electron-dense marker enables us to establish the extent of the extracellular space. The Thorotrast was found not only around the filaments which connect adjacent axonemes, but also in a membrane compartment which surrounds the socket region of each joint. In fact, the Thorotrast seemed almost concentrated in the extracellular space around the socket. Granules of Thorotrast were not present in the axonemes, in the nucleus, or within the space immediately beneath the dense plaque on which the axonemal microtubules insert. By this procedure, then, we were able to determine that the membrane which surrounds the axonemal microtubules is the plasma membrane and that this membrane invaginates all the way to the nuclear capsule. The filaments which connect adjacent axonemes are extracellular; in fact, they are in the seawater that surrounds *Sticholonche*. Further details of these extracellular filaments and their points of connection with the plasma membrane of adjacent axopods will be covered in a subsequent report (4). Since the filaments are extracellular in the seawater and since they do not undergo changes during movement, they cannot be active in the motion observed.

**Effect of Changing the External Environment:** When *Sticholonche* are placed in calcium-free seawater made up as outlined in the Materials and Methods, the organisms continue to move for about 30 min, sometimes longer. In most cases, if the organisms are returned to normal seawater after they have stopped moving, because of the removal of calcium, they lyse indicating that the removal of calcium leads to irreversible damage. If, however, CaCl₂ is added to this seawater, the animals remain viable.

When EGTA (10 mM) is added to normal seawater or if EGTA is added to calcium-free
seawater, or in fact if EGTA and calcium in the same concentrations are added together, the organisms stop beating in 5-15 min. The effect is irreversible, the protists lysing soon thereafter. As mentioned before, when an organism becomes unhealthy the rate of rowing increases. Thus, within a few minutes after the addition of EGTA the rate of rowing increases dramatically. This is followed by a behavior reminiscent of heart muscle fibrillation. During fibrillation the whole organism contracts and then relaxes. The metachrony is lost. Because of the similarities in the motile filaments in Sticholonche and some of the freshwater Ciliates such as Vorticella (20) and Carchesium (2), we placed colonies of the marine peritrichs (organisms similar to the freshwater Carchesium), Zoothamnium pelagicum, in calcium-free seawater. Unlike Sticholonche, these organisms continued their periodic contractions for 1½ h. When they were placed in EGTA, however, the organisms ceased to move and cytolysed within 5 min. Both in Sticholonche and in Zoothamnium, then, the EGTA acts as a toxic agent causing cell death. The toxic effects of EGTA have been reported previously (20), but the mechanism of death is not clear. The use of oxalate was inconclusive; on some occasions the organisms ceased movement, on other occasions they continued to move.

In summary, these experiments demonstrate that extracellular calcium is necessary for viability, yet we cannot prove from these experiments that calcium is a necessary cofactor for the movement.

Membrane Specializations Near the Filaments: As already mentioned, we were able to demonstrate by the use of Thorotrast that there are deep invaginations of the plasma membrane around the base of the axonemes. The cell was incubated in Thorotrast to determine whether the membranous compartments around the base of the axonemes were in reality infoldings of the plasma membrane. The small electron-dense granules are granules of Thorotrast. Note that these compartments contain the Thorotrast, showing that they are continuous with the seawater outside the organism. The micrograph also shows that the fibrils (ef) which connect adjacent axopods are extracellular. Note that the Thorotrast is particularly heavy in the membrane compartment around the socket. \( \times 33,000 \).
membrane. These invaginations follow the contour of each axoneme and, in fact, extend all the way to the nuclear capsule where they surround each socket. Thus the filaments are never more than a fraction of a micron from the cell surface. Upon close examination of the membrane which surrounds the socket we discovered a flattened reticulum attached by a dense substance to the plasma membrane around the socket. Using tannic acid as a mordant we found it possible to resolve the unit membrane structure of the plasma membrane and the reticulum (Fig. 17). The dense material not only connects the plasma membrane to the reticulum but also is present within the reticulum on the side nearest the plasma membrane. The dense material between the reticulum and the plasma membrane in very thin sections can be resolved into small densities and does not appear as a continuous material. The morphology of these membrane specializations becomes more intriguing when one realizes that these specializations closely resemble the transverse tubular system (T system) and its associated sarcoplasmic reticulum, the so-called triad of muscle (12), only in this case it is a diad.

THE LOCALIZATION OF CALCIUM IN STICHOLONCHE: Using the methods of Carasso and Favard (5), we attempted to determine where calcium is located. With these techniques calcium can be identified as small dense crystals. When this technique was applied to Sticholonche, we commonly saw deposits in the mitochondria, in the extracellular space, in the specialized reticulum, and in the nuclear envelope (Fig. 18). There were some granules in the axonemes as well. What is particularly significant is that deposits were conspicuously absent in the regions containing the motile filaments even though within 0.25-0.5-μm numerous deposits could be found. Thus calcium seems to be absent around the filaments but present in the reticulum and in the extracellular space.

THE USE OF THE IONOPHORE X537A TO TRANSPORT CALCIUM INTO THE CYTOPLASM: When we added the ionophore X537A, there was an almost immediate cessation of movement. This was true irrespective of whether the ionophore was added to calcium-free seawater or to normal seawater. A similar reaction occurred in the colonial peritrich (Zoanthidium) which contains a spasmoneme such as that of Carchesium (2) which is known to contract upon the addition of calcium. When the ionophore was added, there was a rapid contraction of the spasmoneme; these organisms did not relax, but remained in the contracted state until lysis took place.

Although these experiments are consistent with a control of motility elicited by calcium in Sticholonche, presumably by liberating intracellular

![Figure 17](image)

**Figure 17** (a) Between the bases of two axopods is an infolding of the cell surface which communicates directly with the surrounding seawater (e). Associated with this infolded region are specialized vesicles (R). × 60,000. (b) Higher magnification of a section of the specialized vesicles from an organism stained with tannic acid. Note that the vesicle (R) is separated from the plasma membrane (m) by dense material. × 190,000.
stores of calcium, they do not prove that calcium is the critical ion since the ionophore transports other ions as well (6).

DISCUSSION

The Relationship between the Microfilaments and the Observed Motility

Using its axopods like oars, Sticholonche literally row through their marine environment. This motion is rhythmic, and the axopods carry out an active stroke followed by a slower recovery stroke which, at least in the dorsolateral and lateral axopods, has an elliptical course. We demonstrated that this fascinating motile event appears to be brought about by microfilaments which connect the plaque onto which the axoneme inserts with the nuclear capsule, since it is only these structures that change during the motile event (they shorten or they lengthen) and it is only these structures that are in the unique position to account for the movement (see below). Neither the microtubules nor the extracellular filaments that connect adjacent axopods appear to be involved in generating the motion. Instead, they have other roles; the microtubules have the role of the oars, the extracellular filaments presumably acting to keep the oars in their specific locations.

There are several points which remain to be elucidated. First, one wonders what variations in the arrangement of filaments or in the arrangement of structures at the hip joint could account for the four different stroke patterns of the axopods. There is a partial explanation for this. A more complete answer is the subject of further work in progress. For the purposes of clarity, let us assume that the dense material consists of a round, flat plate which pivots on a small ball. If we now pull downwards on one edge, the plate will incline; if we pull on the opposite edge, it will incline in the opposite direction: this seems to be the case for the ventral axopods where the filaments appear to insert only on opposite sides of the plate. Yet in the ventral axopods, the axopodial bases are even more specialized. The ball or pivot point is eccentrically positioned; the bulk of the filaments are attached to one side of the plate, with few on the opposite side (illustrations will be published in a separate communication [4]). This distribution appears to be related to the active and passive phases of the movement. Careful examination of grazing sections of this region indicates that...
the filaments on the side of the plate with the greater number of filaments must give rise to the active stroke, the filaments on the opposite side which are fewer in number being responsible for the recovery stroke.

If the ball or pivot point is very small, then motion would be reduced. We presume that the dorsal axopods do not move because each plate contains two or three thin pivot points. And, finally, if the filaments, instead of being distributed evenly around the margin of the plate, are concentrated in certain regions as in the case of the ventral axopods, the motion then could take place in prescribed paths. So far, all we know is that the filaments are unevenly distributed around the dense plates of dorsolateral and lateral axopods. This distribution presumably gives rise to the elliptical movements seen and documented, but the difficulty in orienting oneself when examining thin sections cut through the nuclear capsule has not allowed us to describe the distribution of the filaments in detail.

There is a second point which needs to be explained. What are the variations in the filaments that could account for differences in the relative speeds of the active and recovery strokes and how might some filaments be “turned on” and others “turned off”?

We do not yet have any information that allows us to distinguish morphologically between the filaments used for the active stroke and those used for the recovery stroke. All we know at this point is that the active stroke is correlated with a greater population of filaments than the recovery stroke. Thus, we might say that the speed of contraction seems to be related to the filament number.

**The Nature and Behavior of the Microfilaments**

**The Microfilaments Are Not Actin Filaments**: The microfilaments cannot be actin filaments for three reasons. First, their diameters (20-30 Å) are smaller than those of actin filaments. In thin sections or by negative staining, actin filaments are seen to be 50 Å in diameter (44, 15). If actin filaments are complexed to tropomyosin and/or troponin or to other proteins (43), their size increases; they now measure 70-85 Å in diameter (43, 15). Thus, on the basis of size alone the microfilaments cannot be actin, nor can they be actin filaments complexed to other proteins since complexing would increase the diameter, not decrease it. Secondly, we know that these filaments are capable of shortening up to 10 times their extended length. Actin filaments in all cases studied do not appear to be able to shorten. Instead, as seen by negative staining, by viscosity, by light scattering and by ultracentrifugation, they behave as rather stiff rods. Thus, if observed on a grid or in section, actin filaments present linear profiles. They do not appear to be able to fold, twist, bend sharply, or coil. And, thirdly, the microfilaments do not bind HMM, even though fish and copepod myofibrils in the same reaction vessel do.

**How the Filaments Shorten**: From evidence presented in this report, we have observed the following: (a) When an axopod is maximally inclined the filaments on the side opposite the side with the acute angle present a clear, straight, linear profile, whereas those on the side of the acute angle are not visible appearing instead as finely fibrillar material in thin section. It appears as if the filaments were all piled up on top of each other. (b) When the filaments are partially contracted, a situation intermediate between full extension and maximal contraction (the two states described in a), we see at low resolution that the filament bundle appears to contain a series (three-four) of bands of greater density (see Fig. 19). At higher resolution, one cannot resolve linear filaments in the bands of greater density presumably because they have coiled or spiraled around each other, whereas filaments are clearly resolved in the less dense regions. (c) Close examination of the dense material at the base of the axoneme reveals that it is in the form of small dense rods which appear to be made up of material which is again coiled about itself. These coils are often continuous with the microfilaments which run between the base of the axoneme and the nuclear capsule.

From these observations, then, the filaments in *Sticholonche* are not rigid, linear elements, nor is the movement elicited by the filaments sliding past one another; instead, the filament is shortened by either a coiling, spiraling, or folding of the microfilaments around other filaments or around themselves. The filaments, by such folding, coiling, or spiraling, are capable of shortening to 1/10th of their extended length. We do not see the tubules which Huang and Mazia (20) described in *Stentor* and which were interpreted as being the coil of filaments. The situation seen here, then, is more like that in the spasmone of *Carchesium* as described by Amos (2).
ARE THE MICROFILAMENTS IN STICHLOLONCHE LIKE THOSE DESCRIBED IN PROTOZOA SUCH AS THE VORTECELLIDAE OR THE HETEROTRICH CILIATES SUCH AS STENTOR AND SPIROSTOMUM? In the literature, there are now many examples of protozoa that contain bundles of filaments responsible for contractile events in cells. These filaments often measure 20-40 Å in diameter and, thus, are probably not actin. Examples include Lacrymaria and Tracheloraphis (34, 41), Noctiluca (39), the Acantharia (9), and Didymophyes (16), Spirostomum (8), Stentor (20, 21), and the Vorticellidae, Vorticella, Carchesium, and Zoothamnium (2, 45).

The most extensively studied are the myonemes of Stentor and the spasmonemes of the Vorticellidae (Carchesium and Zoothamnium) both of which are composed of microfilaments 20-40 Å in diameter that characteristically shorten to 50-75% of their resting length. The rate of contraction is extraordinary; in Zoothamnium, for example, the spasmoneme can be shown to contract 15 times faster than the fastest skeletal muscle. The proteins that make up these filaments have been described by Amos and his colleagues (2) and appear to consist of a calcium-binding rubber-like protein whose molecular weight is about 20,000. This protein appears to function in the absence of adenosine 5'-triphosphate by binding calcium (45). In Stentor (21) and Spirostomum (8) as well, calcium without exogenous ATP seems to be important in generating the motion.

There are similarities between the filaments in Sticholonche and those in the ciliates mentioned above. Both filaments have similar diameters and both filaments shorten by folding and/or supercoiling. However, the morphology of the shortened state differs in Stentor, for example, from that described here. In Stentor (20, 21), contraction of the myoneme results in the formation of 10-12 nm tubules. In Sticholonche, we do not see tubules; instead, the filaments come together to form dense bands presumably formed by complex folding or twisting movements. This is consistent with the fact that in transverse section we see dense dots of varying sizes. We also know that in some untreated cells and particularly in cells treated with the detergent Triton X-100, the filaments tend to aggregate into large bundles which appear to be formed by the filaments twisting around one another. We do not know whether these large bundles represent further stages in the
contraction process, stages that are seldom seen in untreated organisms because there are mechanisms to prevent them, or whether these bundles result from the elimination of the attachment site of the filaments to the nuclear envelope due to death or detergent treatment. Similar bundles have not been observed in the spasmonemes and myonemes from other organisms.

There are further similarities between the myonemes and spasmonemes and the filaments in *Sticholonche*. In all these organisms, the filaments shorten to a small fraction of their extended lengths; in *Sticholonche*, for example, to 10% of the extended length. The spasmoneme will shorten to 36% of its extended length (1) and the myoneme in *Stentor* to 25% of its extended length (20). It is interesting to compare these values with the maximum shortening possible for striated vertebrate muscle in which the sarcomeres can change from 3.6 μm at full extension to 1 μm at full contraction, a shortening of 36% (14).

The rates of shortening of the filaments in various systems also merit comparison. In *Carchesium*, the spasmoneme can shorten in 1-2 ms (2); in *Stentor*, contraction occurs in less than 4 ms (31), and, in *Vorticella*, shortening occurs in 4 ms (24). In *Sticholonche*, from data taken from the film, the active phase takes less than 40 ms; in some organisms, it may take somewhat less time. This value was determined in organisms from which the distal ends of the axopods were removed leaving only stubs. Actually, the fastest rate of shortening may be less than this since in the movie (Fig. 3) the event takes place in one frame. In organisms with normal axopods, the rate is slower, however (cf. Figs. 1 and 3). (Of course, it would be better here to express these values in terms of micrometers per second, but this is extremely difficult to do accurately because the amount of filament shortening in *Sticholonche* depends upon the particular axopod that one is observing, and it is difficult to correlate the speed of contraction of one particular group of axopods with a final length of the rest. Because of inaccuracies here, we are expressing these values as the time necessary for shortening.) Despite the method used to measure the speed of shortening, the value we get for *Sticholonche* is at least 10 times longer than the speed observed in the Vorticellidae and in *Stentor* (using the value obtained from organisms with untreated axopods). Furthermore, the recovery stroke in *Sticholonche*, which also seems to be mediated by microfilaments, lasts over 200 ms, or about 50 times longer than that of the ciliate myonemes and spasmonemes. Thus, there exist very large variations in the rates of motility in these different systems.

As will be described in more detail in the next section, in *Stentor*, *Spirostomum*, the Vorticellidae, and in *Sticholonche*, there are membrane vesicles (or a reticulum) near the contractile apparatus which presumably act to mediate ion fluxes, particularly calcium ion fluxes. This fact in itself does not necessarily indicate a similarity between *Sticholonche* and the ciliates, because a morphologically similar calcium-sequestering system occurs in skeletal muscle where the molecular mechanism of contractility is entirely different.

Besides differences in the rates of motility, there is a second major difference between the filaments in *Sticholonche* and the myonemes and spasmonemes in other protists. The filaments in *Sticholonche* appear to insert into the same material into which the microtubules insert and by contracting cause the translation of microtubules. In *Stentor* and in *Carchesium*, microtubules are present as well, yet in *Stentor* and in the Vorticellidae the microtubules and the microfilaments do not directly interact with one another—they are separate systems.

It is clearly premature to make any conclusions about real similarities in the filament systems in *Sticholonche* and in the Vorticellidae and *Stentor* until more is known about chemical similarities in these diverse systems. Thus, at this stage all that can be stated is that the filaments look morphologically similar and that in some ways they behave similarly, i.e. they coil or fold and shorten to a large percent of their resting lengths, yet their relative rates of contraction, at least in untreated cells, are quite dissimilar.

The Control of Metachrony and Filament Contraction

Like the beating of the cilia in a ciliated epithelium or in a ciliate protozoan, the rowing motion of the axopods of *Sticholonche* shows metachronal coordination. Since the plasmalemma invaginates all the way to the nuclear capsule where the "motor" is located, it is tempting to suggest that the metachrony observed is the result of the spread of membrane excitation from the anterior end of the organism to the posterior end in a fashion similar to what is known to occur in *Paramecium* (7) or similar to the spread of excitation down the transverse tubule system of muscle (32).
There is a special reticulum in contact with the invaginated plasma membrane at the hip joint in *Sticholonche*. This reticulum bears a strong morphological resemblance to the triad in skeletal muscle (12). Using a histochemical method, we were able to demonstrate that there are calcium ions in this reticulum, yet there are no deposits amongst the contractile filaments, indicating the absence or low level of calcium in the region containing the filaments. We interpret this observation to indicate that the reticulum maintains a low level of calcium in the region containing the filaments by pumping calcium out of the cytoplasm containing the filaments into the reticulum. During the active phase, as in skeletal muscle, calcium would flux into the cytoplasm. We see no deposit around the filaments, then, because the calcium is sequestered into the reticulum. We also know that an ionophore (X537A) which liberates calcium as well as other divalent and monovalent ions (6) into the cell will cause the almost immediate arrest of movement. These observations plus the fact that in *Stentor*, in *Caerchium* and in *Spirostomum* there is a reticulum which is capable of sequestering calcium ions (5, 8) and the fact that in other ciliates calcium is critical for the functioning of the filaments suggested to us that the controlled influx of calcium mediated by a bioelectric control may in effect “turn on” the motile event in *Sticholonche*. The major difficulty with such a simplified model is to understand how the recovery stroke is mediated since it is slower and requires the “turning off” of some filaments and the “turning on” of others.

We tried to prove that the influx of calcium controls motility in *Sticholonche*. Unlike the situation in skeletal muscle or in ciliates such as *Paramecium* (29, 30), *Stentor* (21) or the Vorticellidae (17, 45) where movement can be induced in glicerinated models by the addition of calcium and thus a positive relationship between calcium and movement can be demonstrated, in *Sticholonche* this is just not possible. To “see” movement adequately in *Sticholonche*, one needs a portion of the axopod. If the membrane is removed wholly or partially, the microtubules break down, except at the point of insertion into the dense material at the hip joint, and thus the axoneme and axopod are lost from view. Attempts at stabilizing the microtubules during lysis were unsuccessful, i.e., with microtubule stabilization medium (10), EGTA, etc. Furthermore, the results of experiments in which the organism was put into calcium-free seawater, into oxalate in seawater (calcium free or not), into EGTA, etc., could not be used to demonstrate an association between calcium and movement because the lack of calcium led to irreversible damage to the organism. Similar observations with the use of EGTA or calcium-free media have been found for *Stentor* (20). Since calcium influences so many cellular properties, not least of which is its action in stabilizing the limiting membrane, and since one can only indicate a direct effect of calcium on the contractile event by using demembranated (partially or completely) models in which contraction is induced by the addition of calcium, we cannot demonstrate conclusively that calcium is inducing the motion effected by the filaments. Our observations, however, are consistent with such an interpretation.

The Importance of Our Observations to Other Systems

We have demonstrated in this report that in *Sticholonche* non-actin-containing microfilaments are attached to a dense material at the tips of the microtubules. These filaments, then, by shortening are capable of moving the microtubules. Thus, we have a system in which the motion is effected by interactions between microfilaments and microtubules, a situation so far not described in any other organism. Since these microfilaments have slender dimensions (20-30 Å in diameter) and can assume a folded or coiled form, they might easily be overlooked, being essentially indistinguishable from the “cytoplasmic ground substance”, particularly if the structure were not so organized as that of *Sticholonche*. For example, particles attached to microtubules could be moved through the cytoplasm by the interaction of the contractile microfilaments with the dense material at the ends of microtubules. Likewise, changes in the distribution of the dense substance which nucleates microtubules (42, 36) could be brought about by such contractile filaments. In fact, there is filamentous material around centrioles and around the dense bodies associated with centrioles in a variety of cell types. Could this material be related to these microfilaments?

By describing just one system in which there is a novel mechanism of motion, i.e. interactions between microfilaments and microtubules, we may perhaps have opened up a whole new realm of possibilities for creating movements in cytoplasm.

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