POLYMERIZATION OF ACTIN

VI. The Polarity of the Actin Filaments in the Acrosomal Process and How it Might be Determined

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ABSTRACT

The polarity of the actin filaments which assemble from the nucleating body or actomere of *Thyone* and *Pisaster* sperm was determined using myosin subfragment 1 decoration. The polarity was found to be unidirectional with the arrowheads pointing towards the cell center. When polymerization is induced at low temperature with concentrations of actin near the critical concentration for polymerization, elongation of filaments occurs preferentially off the apical end. If the sperm are induced to undergo the acrosomal reaction with an ionophore, the polarity of the actin filaments attached to the actomere is the same as that already described, but the filaments which polymerize parallel to, but peripheral to, those extending from the actomere are randomly polarized. These randomly polarized filaments appear to result from spontaneous nucleation. When sperm are induced to undergo the acrosomal reaction with eggs, the polarity of the actin filaments is also unidirectional with the arrowheads pointing towards the cell center. From these results we conclude: (a) that the actomere, by nucleating the polymerization of actin filaments, controls the polarity of the actin filaments in the acrosomal process, (b) that the actomere recognizes a surface of the actin monomer that is different from that surface recognized by the dense material attached to membranes, and (c) that egg myosin could not act to pull the sperm into the egg. Included is a discussion of how the observation that monomers add largely to one end of a decorated filament in vitro relates to these in vivo observations.

KEY WORDS actin filament polarity · acrosomal process · actin nucleation · actomere · actin filament decoration · actin polymerization

When actin filaments interact with myosin, the direction of force production or contraction is determined by the polarity of the actin filaments. Thus, if we know the polarity of the actin filaments (determined by decoration of the filaments with myosin subfragments such as subfragment 1) in the contractile area of a nonmuscle cell, we can predict how that portion of a cell might contract and move. Of obvious interest, then, is to try to determine how a cell establishes a fixed polarity of the actin filaments within a portion of its cytoplasm. Unfortunately, little information on this important topic is available. The most carefully studied system is the brush border or microvillus border of intestinal epithelial cells (10). In that system there is a dense material at the tips of the microvilli which appears to be involved in nucleating the assembly of actin filaments (15).

Since all the filaments that assemble from this dense material have the same polarity, this material seems also to be involved in determining their polarity.

In the last paper in this series (14) another site, the actomere, which appears to nucleate the assembly of actin filaments and thus control their intracellular distribution was described. Unlike the dense material associated with microvillar membranes, this site is a cytoplasmic organelle (14). The actomere is found in echinoderm sperm and appears to function by organizing the polymerization of actin which in turn provides the force and direction for the formation and elongation of the acrosomal process. The polarity of the actin filaments which polymerize from this organelle is important to establish for three reasons. First, if, indeed, this organelle is a nucleating body, one might expect the filaments which polymerize from it to have unidirectional polarity, and thus the nucleating sites might control both intracellular distribution of actin and actin filament polarity. Second, a knowledge of the polarity of the actin filaments in the sperm will enable us to describe what kind of motion we can expect from an interaction of the egg and sperm. To be more specific, can myosin, known to be present in echinoderm eggs, interact with the actin filaments in the acrosomal process to pull the sperm into the egg once fusion has taken place? And third, by determining the polarity of the elongating actin filaments relative to the actomere, we can try to compare what is known from in vitro observations on the addition of monomeric actin largely to one end of a decorated actin filament, so-called biased polar growth (4, 6, 19), to conditions in the cell.

In this paper, then, we describe the polarity of the actin filaments which polymerize from the actomere both in vitro and in vivo using subfragment 1 decoration. We found that the actin filaments which extend from the actomere are indeed unidirectionally polarized and thus the actomere not only behaves as a nucleating material but also establishes the polarity of the actin filaments. What is unexpected is that the nucleating material on membranes and that in actomeres must be of different chemical compositions as these two nucleating sites recognize different surfaces of the actin monomer.

MATERIALS AND METHODS

Organisms and Obtaining Gametes

Thyone briareus were collected by the supply department of the Marine Biological Laboratory, Woods Hole, Mass., and *Pisaster ochraceus* were purchased from Pacific Biomarine, Venice, Calif. These animals were maintained in Instant Ocean Aquarium Systems, Inc., Eastlake, Ohio. The testes were dissected out of each type of organism, minced, and the sperm allowed to ooze out. The residual testes were removed by filtering through cheesecloth and the sperm collected by centrifugation (1,000 g for 5 min). The ovary of *Pisaster* was dissected out of the animal and placed in fresh filtered sea water in a small finger bowl. 1 methyl adenine (final concentration of about 1×10^{-5} M) was added and, within 45 min, eggs were liberated from the ovary. The eggs were separated from the gonads by filtration and collected by centrifugation.

Induction of the Acrosomal Process by Ionophores

Sperm which were suspended in sea water containing 1 mM Tris at pH 8.0 were induced to undergo the acrosomal reaction by the addition of 15 λ of a 1 mg/ml solution of the ionophore X537A to each ml of sperm suspension. The ionophore was dissolved in ethanol and maintained at 0°C in the dark when not being used. The ionophore was obtained from Hoffmann-LaRoche Co., New Brunswick, N. J.

Preparation of Rabbit Muscle G Actin and Subfragment 1 (S1)

1 gm of an acetone powder of rabbit muscle was extracted with 25 ml of CO_2-free water at 0°C for 30 min. The slurry was ground with a mortar and pestle at intervals during this period, then centrifuged at 10,000 g for 10 min and the supernate was isolated. This solution of G actin was used the same day.

S1 was prepared by the procedure of Murray (11) which is slightly modified from that of Lowey et al. (8).

Detergent Extraction, Glycerination, and Decoration with S1

PROCEDURE 1: *Thyone* sperm were demembranated with 0.5% Triton X-100 in 40 mM KCl, 1 mM MgCl₂, and 10 mM Tris buffer at pH 8.0 at 0°C, washed in the above solution which lacked detergent, and incubated overnight at 0°C in 40 mM KCl, 1 mM MgCl₂, and 10 mM Tris at pH 8.0 which contained ~0.3 mg/ml muscle G actin. The next morning the sperm were concentrated in the above salt solution, washed, and incubated at 22°C for 1 h in the salt solution used above to which 3.3 mg/ml of S1 was added. The sperm were then washed in the salt solution and fixed in 40 mM KCl, 1 mM MgCl₂, 10 mM phosphate buffer, 0.2% tannic acid, and 1% glutaraldehyde at pH 7.1.

PROCEDURE 2: *Thyone* sperm were suspended in 0.5% Triton X-100 in 10 mM phosphate buffer at pH 6.4 containing 1 mM MgCl₂ and 60 mM KCl at 0°C to remove the membranes. The sperm were then washed in

1 mM MgCl$_2$, 60 mM KCl, and 10 mM phosphate buffer at pH 7.3, then incubated in the same solution which contained muscle G actin (0.3 mg/ml). The sperm were incubated at room temperature for 10 min in this solution, concentrated by centrifugation, washed briefly in 1 mM MgCl$_2$, 60 mM KCl, 10 mM phosphate buffer at pH 7.3, and then incubated for 30 min at room temperature in S1 with 1 mM MgCl$_2$, 60 mM KCl, and 10 mM phosphate buffer at pH 7.3, washed and fixed in the above solution without S1. The fixing solution also contained 1% glutaraldehyde and 0.2% tannic acid. The pH of the fixative after the addition of tannic acid was 6.8.

PROCEDURE 3: *Thyone* and *Pisaster* sperm were suspended in sea water containing 1 mM Tris at pH 8.0, and then the ionophore X537A was added (15 λ of a 1 mg/ml solution was added to each ml of sperm suspension). 90 s later (for details of why this time was selected, see reference 17) the sperm were suspended in 50% glycerol in 20 mM KCl, 0.5 mM MgCl$_2$, and 5 mM Tris HCl at pH 8.0 at 0°C. After 30 min, the sperm were concentrated and resuspended in 40 mM KCl, 1 mM MgCl$_2$, and 10 mM Tris HCl at pH 8.0 to which 3.3 mg/ml of S1 was added. The sperm were incubated in this solution for 1 h at 22°C, washed in 40 mM KCl, 1 mM MgCl$_2$, and 10 mM Tris HCl at pH 8.0, and fixed in the above salt solution which contained 1% glutaraldehyde and 0.2% tannic acid.

PROCEDURE 4: *Pisaster* eggs and sperm were mixed together and the eggs with their attached sperm were concentrated and suspended in a glycerol solution within 60 s after insemination. The glycerol solution contained 50% glycerol, 25 mM KCl, 0.5 mM MgCl$_2$, and 5 mM phosphate buffer at pH 6.4. The eggs with their attached sperm were incubated for 10 min at 22°C, concentrated, washed in 50 mM KCl, 1 mM MgCl$_2$, and 10 mM phosphate buffer pH 6.4, and incubated in the above salt solution which contained 4 mg/ml S1 for 30 min at 22°C. The sperm were then washed in the above salt solution which contained 1% glutaraldehyde and 0.2% tannic acid.

Electron Microscope Procedures

Extracted decorated models of sperm were fixed in 1% glutaraldehyde (8% stock, Electron Microscope Sciences, Fort Washington, Penn.) and 0.2% tannic acid (Mallinckrodt Chemical Works, St. Louis, Mo., stock No. 1764) as indicated above (1). Fixation was carried out for 30 min. The sperm were then washed in the fixation solution without the glutaraldehyde and tannic acid. Intact sperm were fixed in glutaraldehyde without tannic acid. They were postfixed for 45 min in 1% OsO$_4$ in 0.1 M phosphate buffer at pH 6.0 at 0°C. They were then washed in distilled water and en bloc stained in 0.5% uranyl acetate for 2-3 h at 0°C, dehydrated rapidly in acetone, and embedded in Epon. Thin sections were cut on a Sorvall Porter-Blum II ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.)

with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope.

RESULTS

The Actomere or Nucleating Center of Thyone

In the last paper in this series (14) the fine structure of the actomere was described. It is a body, 0.1 μm in diameter and 0.2-0.3 μm in length, which lies just beneath the acrosomal vacuole. It is composed of 22-25 filaments, each ~70 Å in diameter, which are embedded in a dense matrix. The filaments in the actomere are oriented parallel to the long axis of the sperm such that, if an actin filament was nucleated from one of these 22-25 filaments making up the actomere, these actin filaments would extend directly out into the acrosomal process. Surrounding the actomere is an amorphous material (profilactin, reference 13) which is composed of actin and several other proteins which bind to it. A drawing (Fig. 1) of a thin section through a *Thyone* sperm is included to orient the reader. For more details, see reference 14.

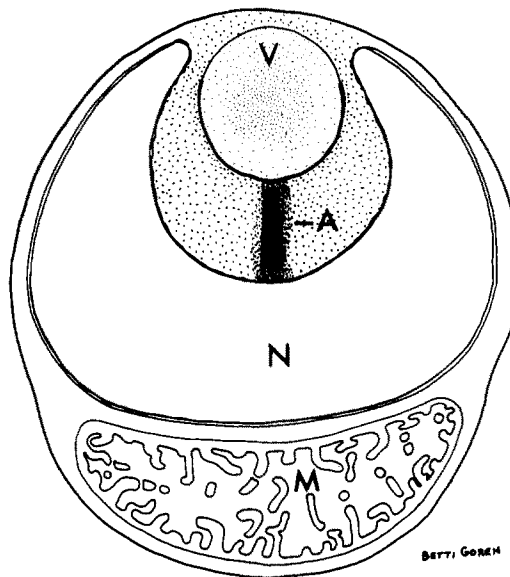To determine the polarity of the actin filaments

FIGURE 1 Drawing of a thin section through a *Thyone* sperm to provide orientation for subsequent plates. Located within the periacrosomal region is the actomere (A). The acrosomal vacuole (V), the mitochondrion (M), and the nucleus (N) are indicated.

which polymerize off the actomere, procedure 1 outlined in Materials and Methods was employed for two reasons. First, when sperm are demembranated at high pH (8.0), the bulk of the profilactin is solubilized, leaving the actomere essentially naked within the nuclear indentation. Thus it is easy to recognize. Second, incubation of sperm for a long period (overnight) at low temperature (0°C) in a concentration of G actin that is only slightly above the critical concentration for polymerization favors polymerization of actin off existing nucleating centers and discourages spontaneous nucleation. The polymerized filaments were then decorated with S1.

In thin sections (Figs 2–4) through the nuclear indentation, actin filaments which have polymerized from the apical surface of the actomere can be identified. All the filaments are decorated; the arrowheads all point towards the center of the nucleus. In most cases, filaments extend only off the apical surface of the actomere as in Fig. 3, although sometimes they extend from both the apical and basal surfaces (Figs. 2 and 4). The polarity of the filaments that extend from the basal end of the actomere is the same as that of those extending from the apical end, namely the arrowheads invariably point towards the center of the nucleus (Figs. 2 and 4). An examination of several hundred micrographs such as Figs. 2–4 reveals that the polarity of the filaments described here is invariant—we have not yet found an exception. Filaments that are not attached to the actomere are rarely seen, indicating that under the conditions employed here spontaneous nucleation seldom occurs.

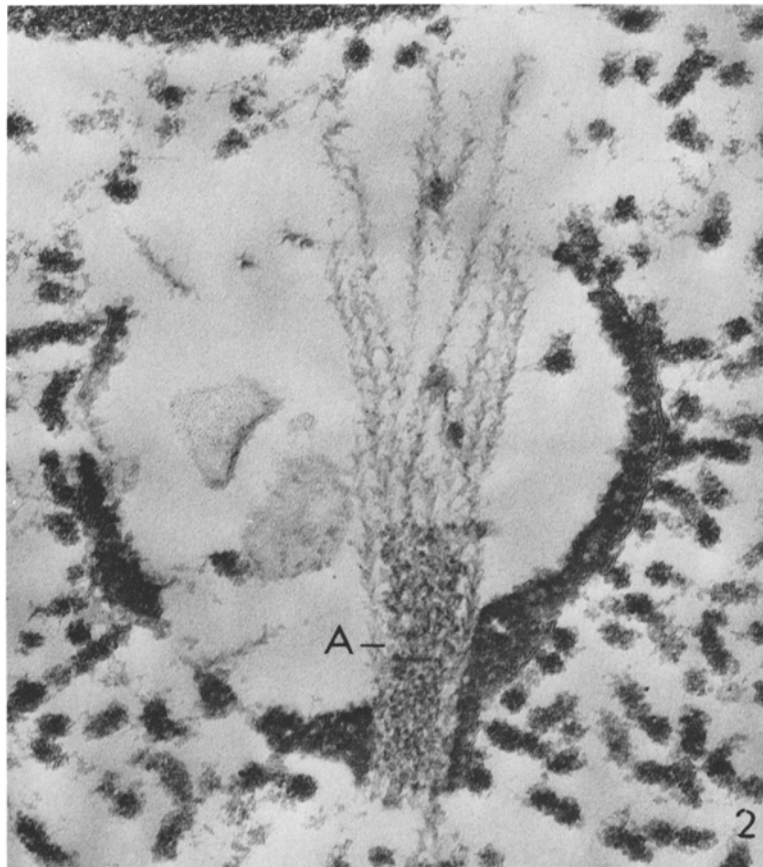A more careful examination of the actomere

FIGURE 2 Thin section through the apical end of a *Thyone* sperm demembranated at pH 8.0. This preparation was incubated in monomeric actin at 0°C overnight, washed, and then decorated in S1 (procedure 1). Of interest are the decorated filaments which extend from the apical and basal surfaces of the actomere (*A*). Note that the filaments in the actomere also decorate with S1. × 80,000.
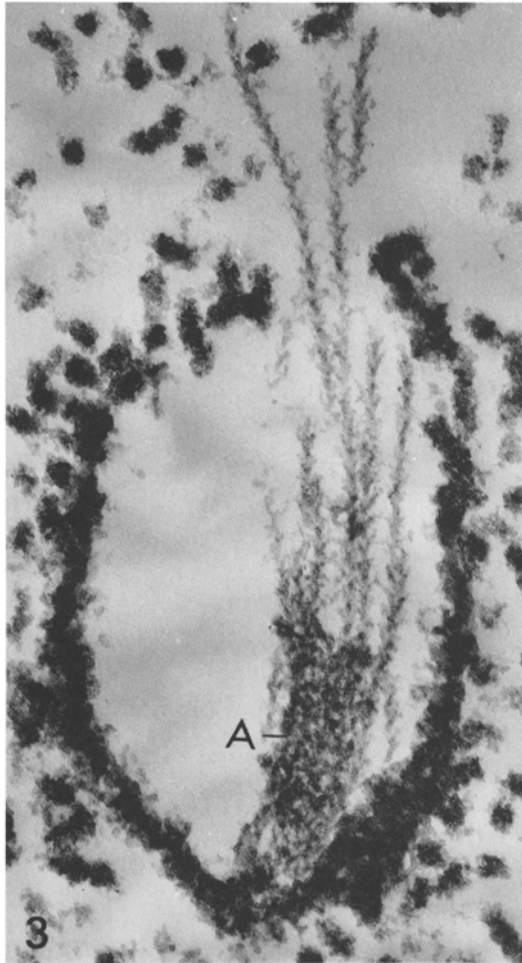
only if incubated in S1 for prolonged periods, it seems probable that the filaments in the actomere are coated with and thus stabilized by an additional protein or proteins which must be removed before decoration can proceed. A closer examination of most actomeres reveals a periodicity of ~350 Å (Fig. 2). This periodicity is probably due to the alignment of the actin filaments in the actomere. Presumably, the additional proteins postulated above which coat the actin filaments in the actomere maintain these filaments in register so that the crossover points of the actin helices of adjacent filaments are in register.

Along the lateral margins of the actomere we often see a single layer of actin filaments which do not appear to be part of the actomere proper, as if these filaments had secondarily attached to the actomere. The polarity of these filaments is the same as that of the filaments which make direct
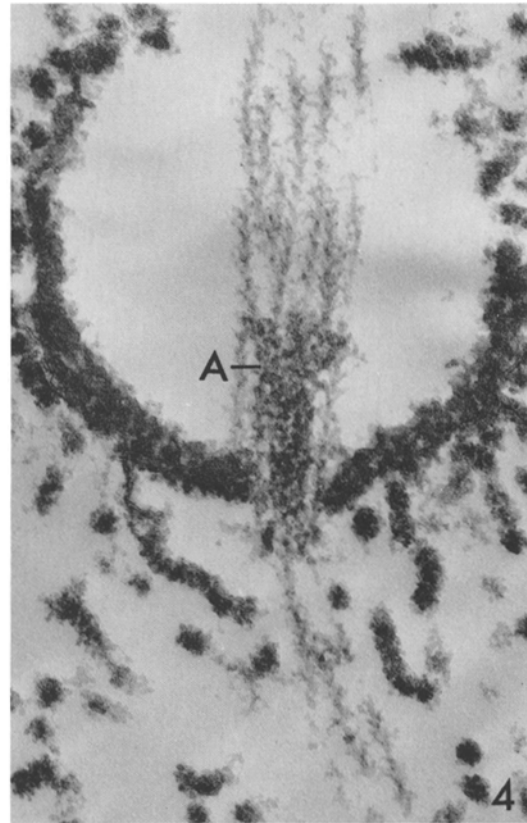

FIGURE 3   Thin section through a portion of a demembranated *Thyone* sperm treated as in Fig. 2. Note the polarity of the actin filaments extending from the actomere (*A*). × 80,000.

reveals that the filaments located in the center of the actomere also decorate (Figs. 2–4), showing the same polarity as the actin filaments that extend from the apical and basal surfaces of the actomere. Thus the filaments which comprise the actomere can be identified as actin filaments. These filaments in the actomere, however, only become decorated if the demembranated sperm are incubated in S1 for long periods such as 1 h at 22°C, whereas the filaments that polymerize from the actomere will decorate after only 30 min in S1 (procedure 2) (Fig. 5). Since the filaments in the actomere before decoration are thicker (70 Å) than purified actin filaments (50 Å) and decorate

FIGURE 4   Thin section through a portion of a demembranated *Thyone* sperm treated as in Fig. 2. Note that the polarity of the actin filaments which extend from either end of the actomere (*A*) is identical. × 80,000.

contact with the apical or basal surface of the actomere. This phenomenon is particularly obvious if demembranated sperm are incubated for a short time (30 min) in S1 (procedure 2). Under these conditions the filaments comprising the actomere do not decorate, yet those attached to the lateral surfaces do (Figs. 5 and 6).

If we demembranate sperm at low pH, i.e., 6.4 (procedure 2), the bulk of the profilactin remains unaltered (13); but when the pH is raised to 7.3 at 22°C in the presence of salt and exogenous G actin is added, both the actin in the profilactin and the exogenous actin will be induced to polymerize rapidly. Under these conditions the actomere is often found translocated from its normal position at the base of the nuclear indentation (Figs. 5 and 6). Actin filaments are now found extending from both the apical and basal surfaces of the actomere (Fig. 6). The polarity of these filaments is unidirectional as is the case when the actin is polymerized slowly (procedure 1).

The Polarity of the Actin Filaments in the Acrosomal Process of Ionophore-Induced Thyone Sperm

As discussed in some detail in an earlier paper (17), the induction of the acrosomal reaction by

ionophores leads to the formation of acrosomal processes which are filled with actin filaments, but which are characteristically only a fraction of the length they would be if induced by eggs or egg jelly. When ionophore-reacted sperm are glycerinated and then the polymerized actin filaments decorated with S1 (procedure 3), the filaments which attach to the apical surface of the actomere display the polarity already described, namely the arrowheads point towards the nucleus (Fig. 7). The filaments which lie parallel to the actomere-inserted filaments, however, appear randomly polarized — the arrowheads of some of them point towards the nucleus, those on others point away from the nucleus. This result is consistent with the speculation of Tilney (14) in which the actomere-associated filaments, by being nucleated, would show unidirectional polarity, whereas the filaments peripheral to those filaments would be the result of spontaneous nucleation and would thus be randomly polarized. In all cases, however, the arrowheads on any one filament all point in the same direction, as one would expect.

The Nucleating Center of Pisaster Sperm

It is clearly important to determine the polarity of actin filaments in the acrosomal process of
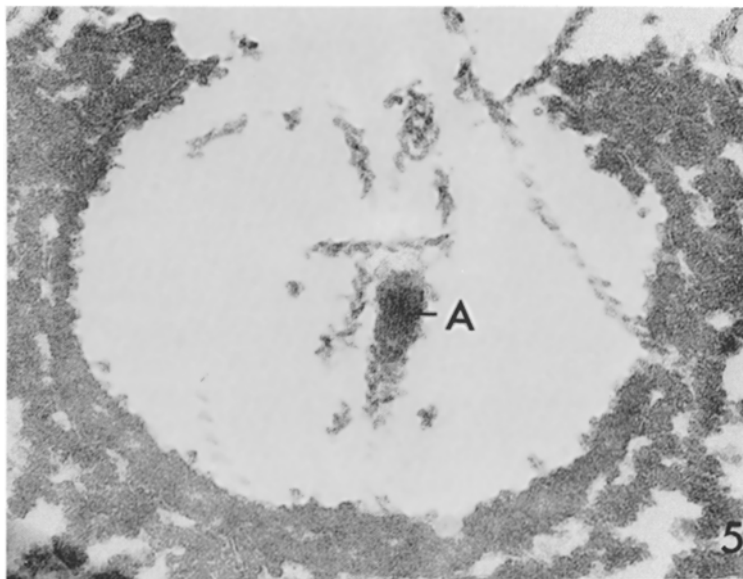

FIGURE 5   Thin section through the apical end of a *Thyone* sperm which was demembranated in Triton X-100 at pH 8.0 (procedure 2). The sperm were then washed and incubated in actin at 22°C for 10 min, then decorated with S1. Of interest is the finding that the filaments making up the actomere (*A*) are not decorated. × 50,000.
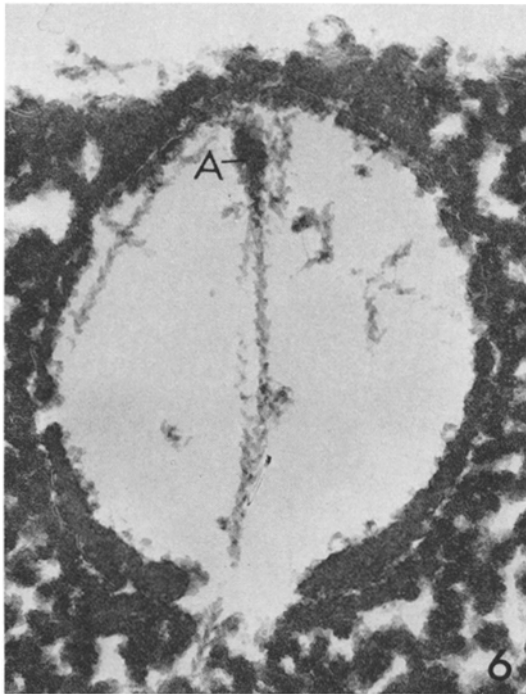
FIGURE 6 Thin section through the apical end of a *Thyone* sperm which was treated as in Fig. 5. The point of interest is that the actomere (*A*) is no longer located at the base of the apical indentation in the nucleus, but has been translocated apically from its original position by the polymerization of filaments off its basal surface. × 87,000.

sperm induced to undergo the acrosomal reaction by natural stimuli such as eggs, particularly since the acrosomal reaction in ionophore-induced sperm is not equivalent to the reaction in sperm induced by eggs (17). However, unlike the gonad of starfish from which eggs can be readily obtained by incubating the gonad in 1 methyl adenine, the gonad of *Thyone* is unaffected by this hormone. Thus the only *Thyone* eggs that can be obtained are from animals which spawn naturally (2). So far, we have not had much luck in finding naturally spawning females; in fact, we have not yet succeeded in obtaining adequate eggs. Therefore, the following experiments were undertaken on eggs and sperm from starfish (*Pisaster*) gametes.

The basic fine structure of the heads of *Pisaster* sperm has been described (17). Of interest to this report is that, unlike the actomere in *Thyone* sperm, the actomere in *Pisaster* sperm and in other starfish sperm consists of a dense material with no filaments embedded within it (see Figs. 8,

9, and 10 of this report; Fig. 2 of reference 16; and Figs. 1, 3, and 4 of reference 3). This is found to be the case irrespective of the plane of section. The dense material has a substructure consisting of many small granules which are often aligned side by side so that we see a series of alternating dense bars separated by a lighter region (Fig. 8). In experiments similar to those reported for the actomere in *Thyone* sperm we (unpublished observations) demonstrated that this dense material in *Pisaster* sperm appears to behave like the actomere in *Thyone* sperm, nucleating the assembly of exogenously added monomeric actin.

The Polarity of the Actin Filaments in the Acrosomal Process of Ionophore-Induced Pisaster Sperm

Most of the actin filaments present in glycerol-extracted *Pisaster* sperm extend apically from the dense material. A careful examination of the decorated filaments (procedure 3) shows that in almost all cases the arrowheads on the filaments point towards the center of the nucleus (Fig. 9). Occasionally, we see filaments with arrowheads pointing away from the nucleus; in these cases the filaments do not make contact with the dense material.

The Polarity of the Actin Filaments in the Acrosomal Process of Naturally-Induced Pisaster Sperm

The arrowheads on the actin filaments in the acrosomal process of *Pisaster* sperm reacted with *Pisaster* eggs (procedure 4) almost invariably point towards the nucleus (Fig. 10). Without exception the filaments which are attached to the dense material show unidirectional polarity with the arrowheads pointing towards the nucleus. In most sperm the dense material remains near the apical surface of the nucleus, with many filaments making contact with its apical surface (Fig. 11), although on rare occasions we find it a short way out the process.

DISCUSSION

The Actomere in Thyone and the Dense Material in Pisaster Behave as Nucleating Bodies which Determine Filament Polarity

In the last paper in this series (14), evidence was presented that the actomere behaves as a
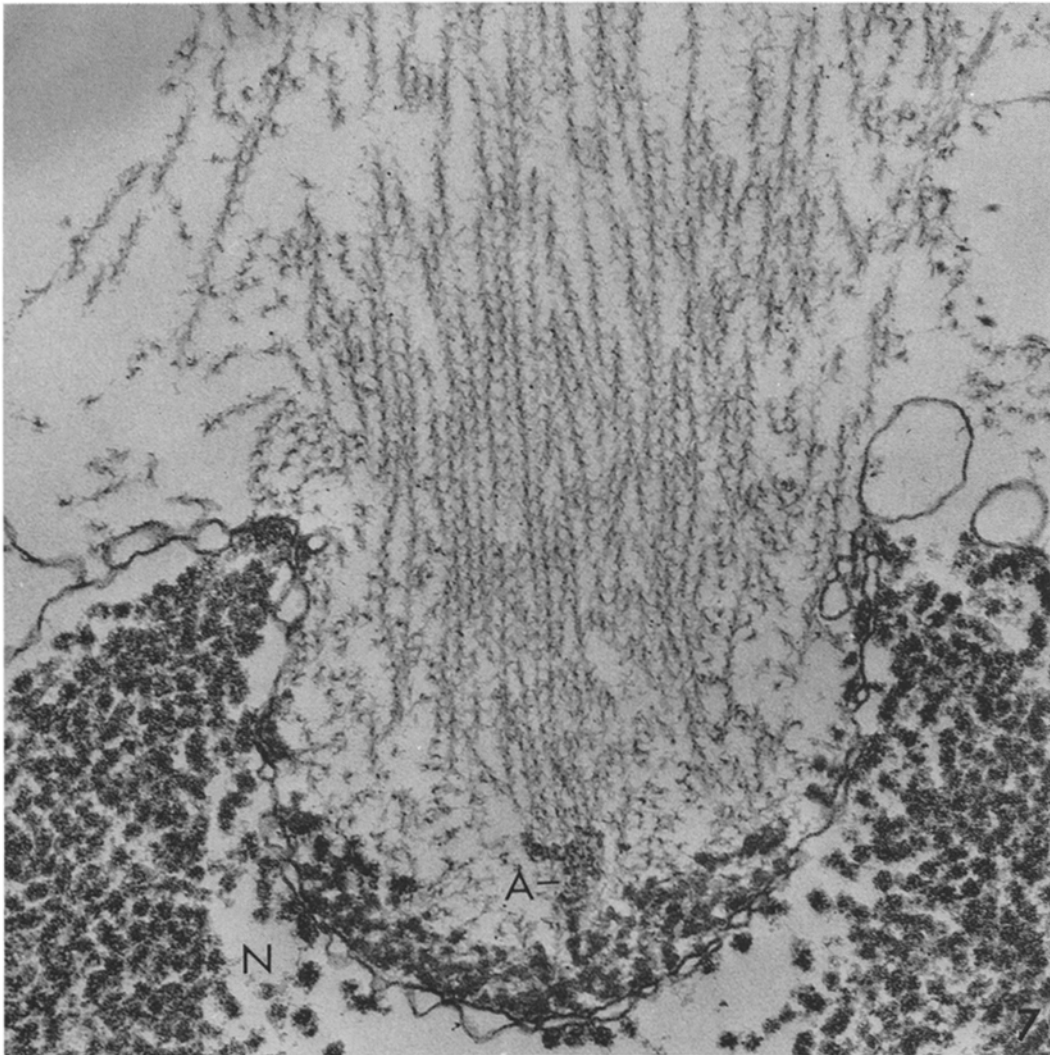
FIGURE 7 Thin section through a *Thyone* sperm which had been induced to undergo the acrosomal reaction with the ionophore X537A, then glycerinated and decorated with S1 (procedure 3). A portion of the actomere (A) is included in the micrograph. Note that the actin filaments which insert into the actomere are polarized with the arrowheads pointing towards the nucleus (N). Those filaments peripheral to the actomere are polarized in either direction. × 64,000.

nucleating body. The results presented in this report support and extend that conclusion. First, when sperm are demembranated at high pH so that the bulk of the profilactin is solubilized leaving the actomere at about its original position (since it sticks to the chromatin) and G actin from muscle is added at a concentration close to the critical concentration for polymerization and at a temperature that induces only a slow polymerization of the actin, essentially the only actin that

polymerizes does so from the actomere. Second, if the actomere in *Thyone* sperm and the dense material in *Pisaster* sperm (we will refer to this material as the actomere as well for expedience as the differences are irrelevant to this report) behave as nucleating bodies, then one would expect filaments extending from these bodies to be unidirectionally polarized. We demonstrated in this report that they are. Third, it was suggested in the last paper in this series (14) that, if ionophores

are added to sperm at high external pH's, the actin would be explosively released from its binding proteins so that spontaneous nucleation of the actin filaments would occur along with nucleated polymerization from the actomere. Thus one would predict that whereas the polarity of the actin filaments attached to the actomere would be unidirectional, that of the peripheral filaments would be random. We illustrated that this is, in fact, the case. Thus the actomeres of *Thyone* and *Pisaster* sperm seem to behave as nucleating bodies which also determine filament polarity.

Similarities and Differences between the Nucleating Bodies of Echinoderm Sperm and those from Other Nonmuscle Cells

The similarity in function between the actomeres in *Thyone* and *Pisaster* sperm and the dense membrane-associated material in microvilli is striking. Both appear to nucleate actin filament assembly and control the polarity of the actin filaments. However, there are two very important differences. First, the actomere is a cytoplasmic structure, whereas the dense material that nucleates actin polymerization in microvilli is membrane bound. Second, although the polarity of the actin filaments relative to the cell center is the same for filaments nucleated from the actomere and for filaments nucleated by the dense material attached to the tips of microvilli (see Fig. 12), the direction of elongation of these filaments is different in these two systems. In the case of the microvilli the filaments are growing towards the cell center; and in the case of echinoderm sperm the filaments are growing away from the cell center towards the membrane. Since the polarity of the filaments is the same, yet the direction of elongation is different, this means that the nucleating material in each case must be binding to a different part of the surface of each actin monomer. This point is most readily appreciated by an examination of Fig. 13 in which the actin monomer is depicted as a heart. In the case of nucleation from the sperm actomere the pointed end of the heart is recognized by the nucleating material, whereas in the case of nucleation from the dense material at the tips of microvilli the cleaved end of the heart is recognized by the nucleating material.

One of the reviewers of this manuscript felt that we should emphasize that the actomere of *Thyone* sperm has preformed filaments within it, whereas the dense material (actomere) of *Pisaster* sperm

and the dense material at the tips of microvilli do not. Although this is true, this difference may be unimportant in view of the fact that the actin filaments in the actomere of *Thyone* sperm must have been polymerized at an earlier stage in spermiogenesis, presumably being nucleated and organized by the same dense material that surrounds them in the mature sperm. One does wonder, then, why there are preformed filaments within the actomere of *Thyone*, but not in *Pisaster* sperm or associated with the membrane before microvillar elongation. Perhaps a preformed cluster of filaments leads to a more efficient and therefore more rapid polymerization of actin which must occur in *Thyone* sperm because of the length of the process and the rapidity of the reaction.

How Might the Polymerization of Actin from the Actomere be Related to the In Vitro Observations on Actin Polymerization from One End of a Decorated Filament? What Does Such a Consideration Tell Us About How the Acrosomal Process Develops?

Three reports have been published recently which demonstrate that actin monomers add preferentially to one end of a decorated actin filament (4, 6, 19). These reports are based upon experiments carried out using short segments of decorated actin filaments as the nucleating source. The preferred end corresponds to the shaft end of the decorated filament or that end which would be attached to the Z line of muscle. This, in fact, is the end of the actin filaments in the actomere to which monomers prefer to add in vitro as we demonstrated in this report by incubating demembranated *Thyone* sperm in low concentrations of monomeric actin. Since the filaments attached to the actomeres of *Thyone* and *Pisaster* sperm were decorated after monomer addition, not before, it could not be the S1 decoration itself which decides which end is preferred. Thus our experiments amplify the in vitro observations of others by demonstrating that prior S1 decoration does not change the preferred end for addition.

In vivo, monomers could add to the distal end (the preferred end), the proximal end, or even to the actin filaments at varying positions along their lengths. The last possibility seems unlikely as addition of monomers to the middle of a filament would require breaking at least three bonds for
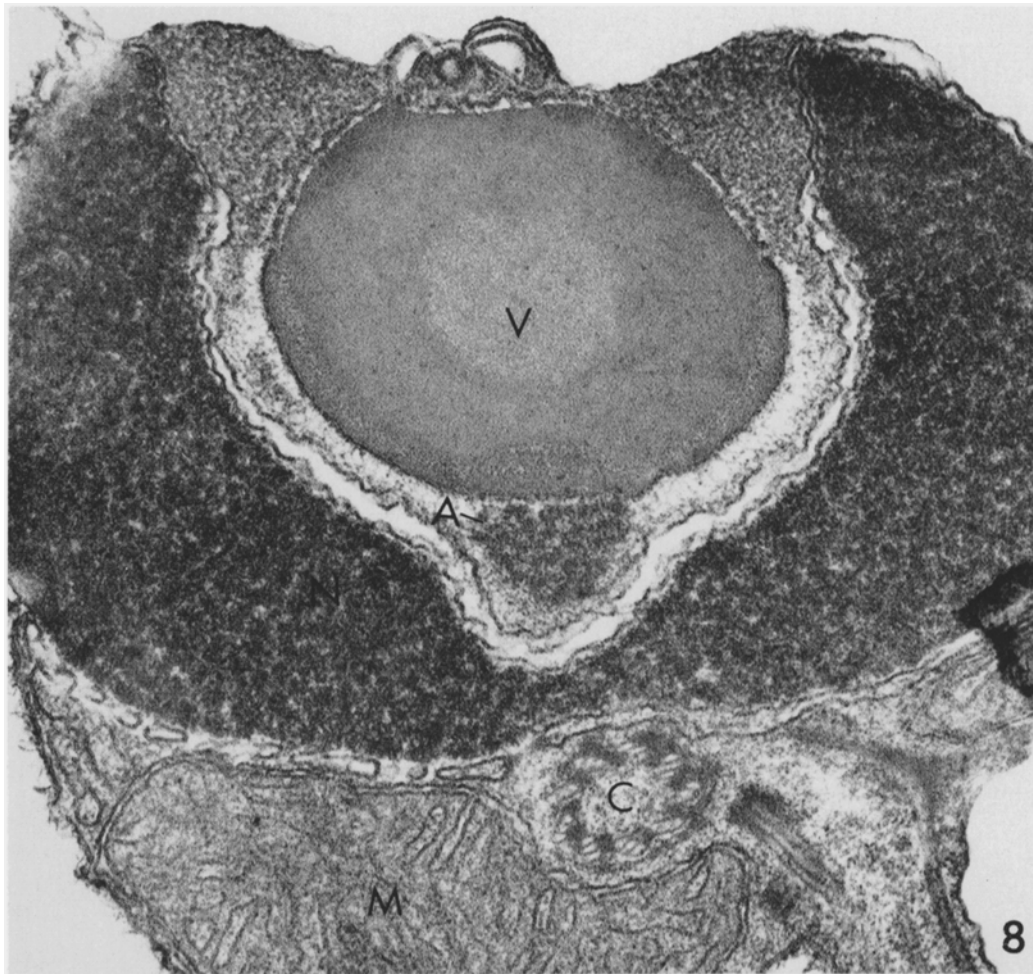
FIGURE 8 Thin section through the apical end of a *Pisaster* sperm. The acrosomal vacuole (*V*), the nucleus (*N*), the centriole (*C*), a portion of the mitochondrion (*M*), and the actomere (*A*) are indicated. Note that the actomere of *Pisaster* consists of small dense granules arranged into rows. × 80,000.

each monomer added. Furthermore, this does not occur when the filaments are decorated with S1 before monomer addition (4, 6, 19). In the following discussion we will try to determine, on the basis of the available evidence, whether the monomers would add to the distal end or the proximal end in vivo. Let us first examine the situation if the actin monomers add exclusively to the distal tip, the preferred end for addition of monomers. If this is the case, then the monomers would have to diffuse from where they are stored in the profilactin region out along the extending actin filament bundle to the tip of the process (Fig. 14). Knowing the length of the acrosomal process (90 μm) and the rapidity of the reaction (10 s), one

wonders if there is sufficient time for the monomers to diffuse out this distance. If not, then we must either look for a mechanism to facilitate diffusion of monomers to the tip, or conclude that the monomers add to the elongating filaments at their base. Therefore, we must now determine how long it will take for monomers to diffuse out to the tip of a 90 μm long process.

We model the situation as follows (Fig. 15). A reservoir of monomer at concentration C_0 serves as a source from which monomers diffuse to the right along the X axis, effectively into a sheet. The freely diffusing monomers are subject to polymerization which we depict as a rapid absorption into fixed sites or holes distributed along the X

FIGURE 9 Thin section through the apical end of a *Pisaster* sperm which had been induced to undergo the acrosomal reaction with the ionophore X537A, then glycerinated and decorated with S1 (procedure 3). The actin filaments which extend from the actomere (A) are unidirectionally polarized—they all point towards the nucleus. $\times$ 64,000.

axis. Polymerization is treated as a fast process compared to the diffusion. The solution to the diffusion equation with these boundary conditions has been given by Hermans (5). A moving boundary of monomer is generated at the front of the polymerized sites or occupied holes. As it extends, the monomers attach to the tip of the elongating filaments and their positions must be filled by more monomers diffusing from the profilactin region (Fig. 14). The position of this boundary, ξ, is proportional to $\sqrt{Dt}$ where t is time:

$$\xi = 2Z\sqrt{Dt}, \tag{1}$$

with D the diffusion constant and Z a constant which is chosen to satisfy the condition that $C = 0$ for $X > \xi$, which is:

$$Ze^{Z^2}\,\mathrm{erf}\,Z = 1/\sqrt{\pi}(C_0/C), \tag{2}$$

where erfZ is the error function. Solutions of equation 2 for Z, given C_o and the free concentration of monomer, C, are tabulated in Hermans' article (5); from these the position of the boundary can be obtained using equation 1.

Consider the case in which the concentration of monomers at the tip, C, is at the critical monomer concentration for actin polymerization while the concentration, C_0, is 100 times higher; let D = 5 × 10^{-7} cm^2/sec or the diffusion constant for hemoglobin, and put ξ = 90 μm, the final length of the acrosomal process. Equations 1 and 2 yield t = 12 s for this to happen, assuming that diffusion occurs in a medium with the viscosity of water. This seems superficially reasonable, but, in fact, the time scale of the process depends critically on

the ratio C_0/C. If actin monomers really are involved in a diffusion process, at any stage C_0 < C, if C is the critical monomer concentration. If C_0 exceeds this value, spontaneous nucleation should occur and instead of seeing the formation of a needle-like process 90 μm long, one would see a short, fat pseudopod (17). But, if C_0 = C in equation 2, with ξ = 90 μm and the same diffusion constant as before, t = 100 s, much longer than observed. There are two ways out of this dilemma: (a) the diffusing substance is not actin monomer, but some inactivated form of it coming from the profilactin region; or (b) the diffusion of monomers is strongly facilitated, for example, by carrying monomers along the extending membrane. This would reduce the effective
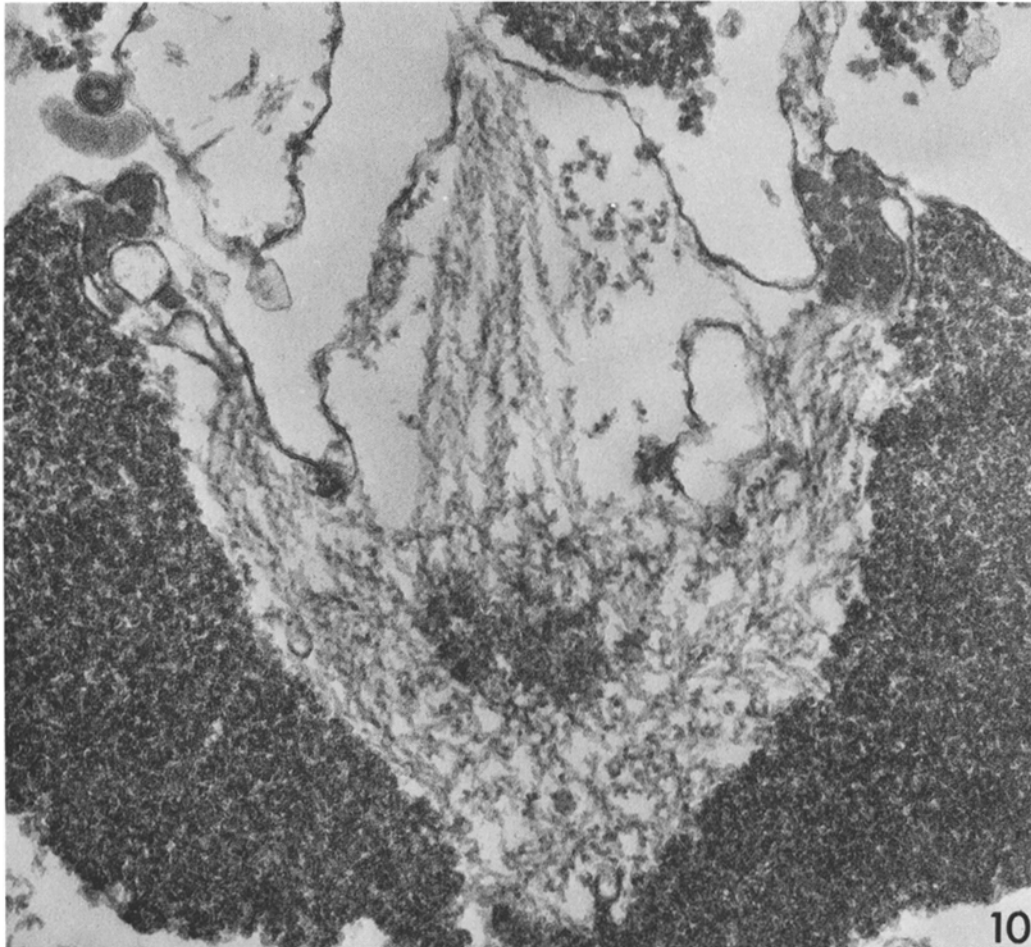

FIGURE 10   Thin section through the apical end of a *Pisaster* sperm induced to undergo the acrosomal reaction with a *Pisaster* egg, then glycerinated and decorated with S1. Note the polarity of the actin filaments attached to the actomere. × 92,000.
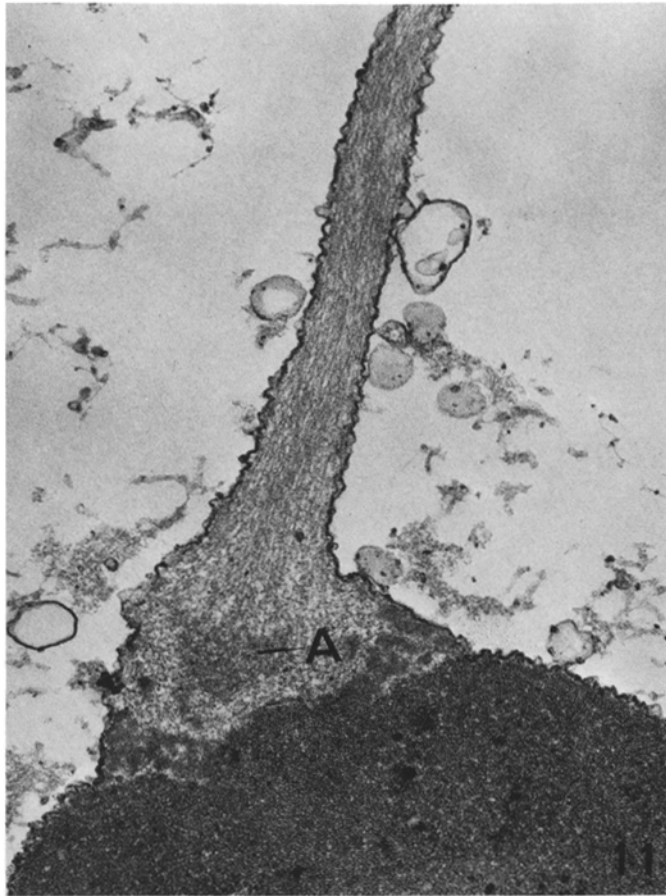
FIGURE 11 Thin section through the apical end of a *Pisaster* sperm induced to undergo the acrosomal reaction with a *Pisaster* egg. Of interest is the finding that the actomere (A) remains at the base of the acrosomal process. × 50,000.

distance that monomers travel by a factor proportional to $(d/dt)\xi$, and could cut the time by a factor of 10 in the ideal case where each monomer is dragged maximally. It seems unlikely that the ideal case could be realized in a cell. Thus we conclude that addition of monomers to the process exclusively at the growing tip cannot be responsible for the rapid elongation of the process if free diffusion of monomers plays a role. We cannot rule out more complex mechanisms such as described above; the simplest one, however, is inadequate.

Perhaps the actin monomers add to both ends of the filaments in the actomere. Initially, they would add to the apical end of the actin filaments (the preferred end), but as the distance increased from the free monomer pool in the profilactin region to the end of the filaments at the tip of the process, diffusion would limit further addition to that end. Now, monomers would tend to add onto the basal end of the filaments in the actomere, or the end nearest the center of the nucleus. In such a model the actomere would have to be translocated into the process, its distance up the process being a function of the diffusion rates. One test of this model would be to examine the position of the actomere upon completion of the acrosomal reaction. When *Pisaster* sperm which had been induced to undergo the acrosomal reaction with eggs were examined, it was clear that the actomere generally remained near the nucleus (Fig. 11). Due to the difficulty in obtaining eggs from *Thyone*, we have not yet determined the location of the actomere in naturally induced *Thyone*
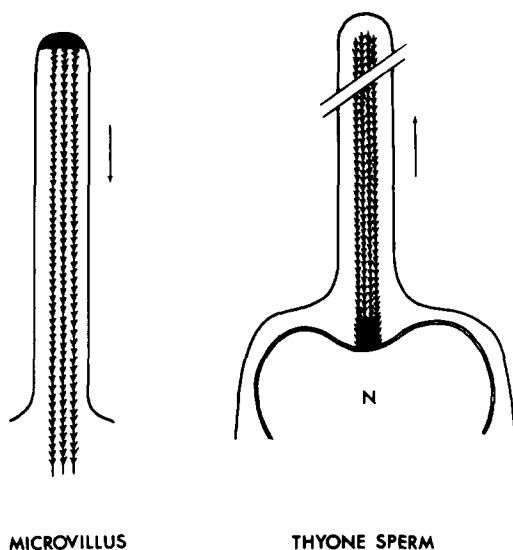
MICROVILLUS   THYONE SPERM

FIGURE 12   Drawing illustrating the polarity and at-
tachment of the actin filaments in *Thyone* sperm and
microvilli. The arrows indicate the direction of elonga-
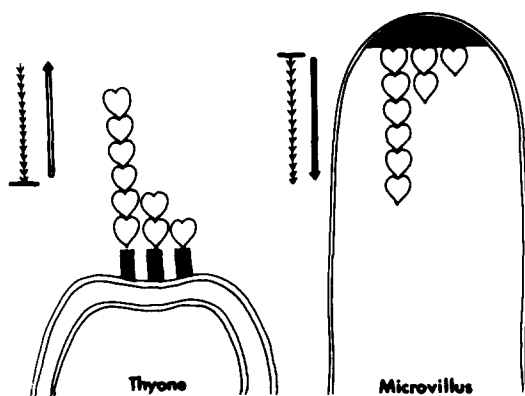tion of the filaments. N = nucleus.

rally induced *Thyone* sperm the actomere remains
near the nucleus. Thus this mechanism also seems
unlikely.

How then can a process 90 μm long form in 10
s? One possibility is that the insertion of mono-
mers occurs at the point of contact between actin
filaments in the actomere of *Thyone* sperm and
the actin filaments which extend from it. In this
model the dense material in the actomere may
behave as an enzyme to facilitate the incorpora-
tion of the monomers into the actin filament at its
basal connection with the actomere. In essence,
then, the actin filament becomes slightly disasso-
ciated from the actomere so that monomers could
add onto it, then it glues back to the filaments in
the actomere. Another possibility is that the ex-
tension of the acrosomal process is carried out by
a telescoping of actin filaments past one another.
All that is required of such a mechanism is the
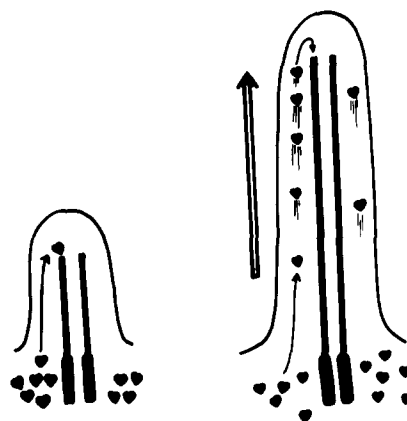presence of cross-linking molecules (macromole-

FIGURE 14   Drawing illustrating the situation if mono-
mers (hearts) are to add to the elongating tips of the
actin filaments nucleated from the actomere.

FIGURE 13   Drawing of the apical end of the nucleus
and actomere of a *Thyone* sperm and the tip of a
microvillus. Actin monomers, indicated by the hearts,
are seen to add onto the actomere and the dense
nucleating material at the tip of the microvillus. The
polarity of the filaments in both cases is indicated and
the direction of polymerization is shown by the arrows.
Of interest is that these two nucleating sites must recog-
nize different parts of the surface of the actin monomers
illustrated here by the hearts.

sperm, but from our experiments using iono-
phores (13) it is not seen to be translocated out
into the process; and Summers (personal commu-
nication) informs us that in his pictures of natu-

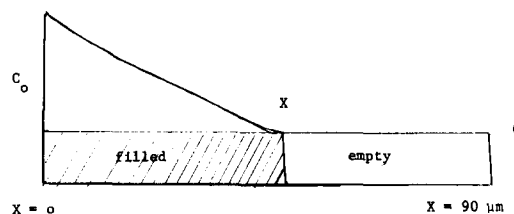Trapping of monomer in "holes" as they diffuse.

FIGURE 15   The relationship derived by Hermans (5)
for the diffusion of a moving boundary in which the
monomer is progressively trapped in "holes" as it dif-
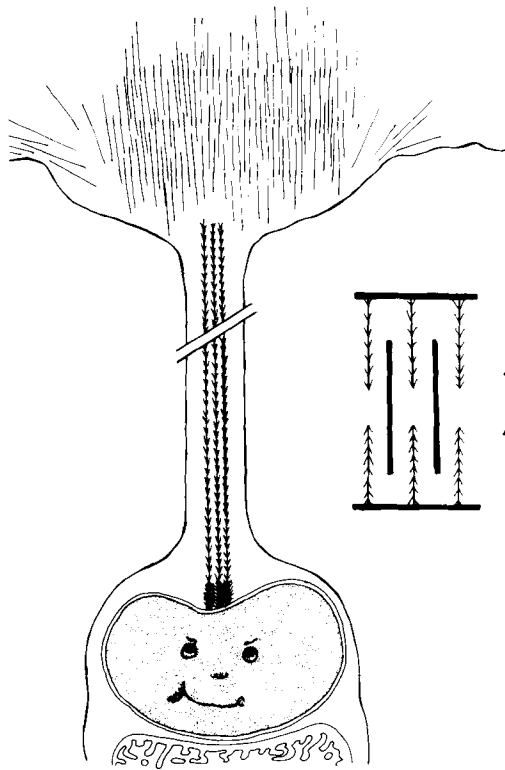fuses forward is outlined here. For details see text.

FIGURE 16 A diagrammatic rendition of the interaction of a sperm with the surface of an egg. The polarity of the filaments in the acrosomal reaction is indicated. The polarity of the actin filaments in a muscle sarcomere is indicated on the right. The arrows point out the direction of the contraction of the sarcomere. Knowing the polarity of the actin filaments in the acrosomal process will tell us if egg myosin could, by interacting with the actin filaments in sperm, pull the sperm into the egg. It can't.

How Does the Sperm Get into the Egg? Can Egg Myosin, by Interacting with the Actin Filaments in the Sperm, Translocate the Sperm towards the egg?

In a detailed and elegant study the Colwins (2) described, in a sequence of phase contrast micrographs, how *Thyone* sperm are transported towards the egg after the acrosomal process has penetrated the egg jelly. They showed that shortly after the tip of the process has made contact with the surface of the egg, a cone of cytoplasm produced from the egg begins to extend outwards along the acrosomal process. Yet, as this cone moves outwards, the whole sperm glides towards the egg. Ultimately, the entire content of the sperm is incorporated into the cytoplasm of the egg, with the plasma membrane of the sperm existing as a patch on the surface of the egg. An interesting question is: What provides the force to move the sperm towards the egg as the cone extends outwards? Is this force produced by the sperm, the egg, or both? More specifically, does myosin which is known to be present in echinoderm eggs (9) interact with the actin filaments in the acrosomal process, once the membrane at the tip of the process has fused with the plasma membrane limiting the egg, in such a way that the sperm is pulled into the egg? If this contraction occurs by the same mechanism as that in skeletal muscle or in the isolated brush border (10), the answer must be "no," because the bulk of the actin filaments in the acrosomal process of the sperm are polarized in the wrong direction. This point is shown diagrammatically in Fig. 16. It is as if the tip of the acrosomal process were located at the "Z" line of muscle. Thus the actin filaments in the acrosomal process are polarized such that if a myosin filament were to interact with them, it would have to be located at the position occupied by the sperm nucleus; a myosin filament in the egg could not pull the actin filaments in the sperm into it. Admittedly, there are, in naturally induced acrosomal processes, a few filaments which are polarized in the appropriate direction for contraction, but this is not consistently the case as thin sections through acrosomal processes illustrate those with all the filaments polarized in the opposite sense for contraction. Since eggs have elaborate mechanisms to insure that only one sperm gets in, it is extremely unlikely that fertilization would be dependent upon a few spontaneously nucleated filaments which do not occur in all

cules?) that cross link only filaments of the same polarity. In this telescoping model, new filaments would be constantly forming due to spontaneous nucleation as they would be closer to the source of monomer (profilactin) and thus diffusion would not limit the extent of the process. Individual filaments would not extend all the way from the actomere to the tip of the process. At this moment we have no evidence as to the length of individual filaments, data which are difficult to obtain. In both these models, addition of monomers to the tip could and probably does occur along with either telescoping of the filaments past each other or addition of monomers between the actomere filaments and the filaments that extend from them.

sperm cells. How, then, do sperm move towards the egg as the fertilization cone extends outwards? One can devise mechanisms in which sperm myosin, by being membrane bound or by being located near the sperm nucleus, could, in essence, pull the egg towards the sperm once membrane fusion has occurred. Alternatively, there may be interactions between the filaments in sperm and filaments of opposite polarity in the egg such that the filaments in the egg might be translocated by egg myosin. This latter possibility is particularly interesting as the cytoplasm at the point of contact between the sperm and the egg undergoes an intricate local rearrangement. At this point of contact the egg cytoplasm becomes packed with enormous numbers of filaments oriented primarily parallel to the acrosomal process (18). Further characterization of this "ouch" response of the egg is in progress.

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