

## POLYMERIZATION OF ACTIN

### V. A New Organelle, the Actomere, That Initiates the Assembly of Actin Filaments in *Thyone* Sperm

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#### ABSTRACT

Between the acrosomal vacuole and the nucleus is a cup of amorphous material (profilactin) which is transformed into filaments during the acrosomal reaction. In the center of this cup in untreated *Thyone* sperm is a dense material which I refer to as the actomere; it is composed of 20–25 filaments embedded in a dense matrix. To visualize the substructure of the actomere, the profilactin around it must be removed. This is achieved either by demembrating the sperm with Triton X-100 and then raising the pH to 8.0, or by adding ionophores to intact sperm at pH 8.0. Under these conditions, the actomere remains as a unit while the rest of the profilactin is solubilized or polymerized. When demembrated sperm are incubated under conditions in which the actin should polymerize, filaments grow from the end of the actomere: the actomere thus appears to behave as a nucleating body. This observation is strengthened by experiments in which untreated sperm are incubated in seawater or isotonic NaCl at pH 7.0 and the ionophore X537A is added; in this case, only a partial polymerization of the actin occurs and the acrosomal vacuole does not fuse with the cell surface. The actin filaments that do form, however, are attached to the apical end of the actomere. In fact, the elongating filaments push their way into and frequently through the acrosomal vacuole. Thus, it appears that the sperm organizes the actin filaments by controlling their nucleation. My model is that the cell controls the amount of unbound actin such that it is slightly above the critical concentration for polymerization. Then, spontaneous nucleation is unfavored and polymerization would proceed from existing nuclei such as the actomere.

**KEY WORDS** actin polymerization · sperm ·  
nucleation · acrosome reaction · actomere

One of the most fascinating aspects in the assembly of structural proteins such as microtubules and actin filaments in cells is the control of their distribution, for ultimately the factors involved in this control regulate motility, cell shape, intracel-

lular transport, and secretion. Although many actin filaments appear randomly distributed in cells or in large regions of cells, actin filaments are often seen attached to specific regions of the cell, i.e., to the “Z” line in skeletal muscle, to the dense bodies in smooth muscle, and to membranes in many nonmuscle cells. Whether this attachment occurs because these sites nucleate the

assembly of the actin filaments or whether the actin filaments attach to these sites after assembly has taken place, or both, is undetermined. For example, how actin filaments with the correct polarity form in developing skeletal muscle remains obscure (14). I know of only three cases in nonmuscle cells in which there is evidence demonstrating that the actin filaments grow from specific sites, i.e., in *Mytilus* sperm, in *Limulus* sperm, and in the formation of intestinal microvilli (the evidence has been summarized in reference 21). It is interesting that in two of these cases, *Mytilus* sperm and microvilli, the polarity of the actin filaments (as defined by the mode of attachment of the proteolytic fragments of myosin to the actin filaments) is known, and one can tentatively conclude that nucleation and polarity determination may be under similar controls in these systems.

Actin filaments can also be attached to membranes along their lengths and/or to one another by macromolecular bridges (3, 6, 9, 16, 24). The cross-linking of filaments to membranes can, in fact, lead to a nonrandom distribution of filaments. Although the polarity of the actin filaments in the bundle is known in some systems, e.g., in the streaming regions of plant cells (8, 11), in retinal cells (1), and in clotting amoebocytes (5), there is no information on how such polarized bundles of filaments become organized. Such an arrangement could be determined if the bridging macromolecule recognizes the polarity of the actin units in the filaments.

I have been exploring the acrosomal reaction of certain invertebrate sperm as an experimental system with which to study motility in nonmuscle cells. This fantastic reaction consists of the rapid formation (10 s) of a process which can exceed 90  $\mu\text{m}$  in length; it has been suggested that the force for the production of this process is related to the explosive polymerization of actin (22). Before the acrosomal reaction, the actin is stored in the sperm in an insoluble complex by being bound to other proteins. This complex appears as an amorphous material that takes the form of a cup; I have referred to this cup of amorphous material as profilamentous actin (profilactin; reference 18). Attempts at the induction of polymerization of profilactin *in vitro* were only moderately successful. What is interesting is that polymerization occurred only from the center of the cup in relation to some filamentous material which was more electron dense than the material in the rest of the cup. I speculated that these dense filaments

(core filaments) might nucleate the assembly of the actin, a conjecture originally proposed by Colwin et al. (2) and Summers et al. (15).

In this paper, I will describe the dense "core filaments" in more detail in preparations from which the bulk of the profilactin has been removed either by solubilization or polymerization. If the profilactin remains intact, there is so much enmeshing material present that these core filaments are difficult to resolve. I will, then, demonstrate experimentally that the core filament bundle orders the assembly and direction of polymerization of the actin filaments in the acrosomal reaction in a manner analogous to the basal body of a cilium or a flagellum.

## MATERIALS AND METHODS

### *Biological Material*

*Thyone briareus* were obtained from the supply department of the Marine Biological Laboratory (Woods Hole, Mass.) and maintained in running seawater, or for short periods in an Instant Ocean tank (Aquarium Systems, Inc., Eastlake, Ohio), courtesy of Drs. S. Inoué and H. Sato.

### *Source of Chemicals*

X537A was graciously supplied by Hoffman-La Roche, Inc. (Plainfield, N. J.). Triton X-100 was obtained from Schwarz/Mann Div., Becton, Dickinson & Co. (Orangeburg, N. J.), and glutaraldehyde (8% stock) was purchased from Electron Microscopy Sciences (Fort Washington, Pa.).

### *Electron Microscope Procedures*

Sperm were fixed by the addition of sufficient glutaraldehyde to the last solution in which the sperm were placed to make the suspension 1%. Fixation was carried out for 30 min. The conditions used are mentioned in the figure legends. The sperm were washed briefly and then postfixed in 1%  $\text{OsO}_4$  and 0.1 M phosphate buffer at pH 6.0 at 0°C for 45 min. The sperm were then rinsed three times in cold distilled water and en bloc stained with 0.5% uranyl acetate for 1–3 h at 0°C, dehydrated rapidly in acetone, and embedded in Epon 812. Thin sections were cut with a diamond knife on a Porter-Blum II ultramicrotome (Dupont Instruments-Sorvall, DuPont Co., Wilmington, Del.), stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope.

## RESULTS

### *Untreated Sperm*

The fine structure of untreated *Thyone briareus* sperm has been described by Colwin et al. (2) and

in the second paper in this series (17). I will summarize here only what is relevant to a discussion of how the actin filaments may be ordered in the acrosomal process. For additional information about the structure of *Thyone* sperm, the reader should consult the above references.

The acrosomal vacuole lies within an indentation in the apical end of the nucleus; between it and the nucleus is the profilactin which takes the form of a cup (Fig. 1). Of particular interest to this report is the electron-dense material in the

center of the cup of profilactin which consists of a series of linear elements extending from the basal limits of the acrosomal vacuole membrane towards the nuclear envelope (Fig. 1). Although this material can be easily differentiated from the rest of the cup, its substructure is difficult to resolve due to the high density of the surrounding material. I will refer to this material as the actomere. The contents of that part of the acrosomal vacuole immediately above the actomere is less dense than the material in the rest of the vacuole.

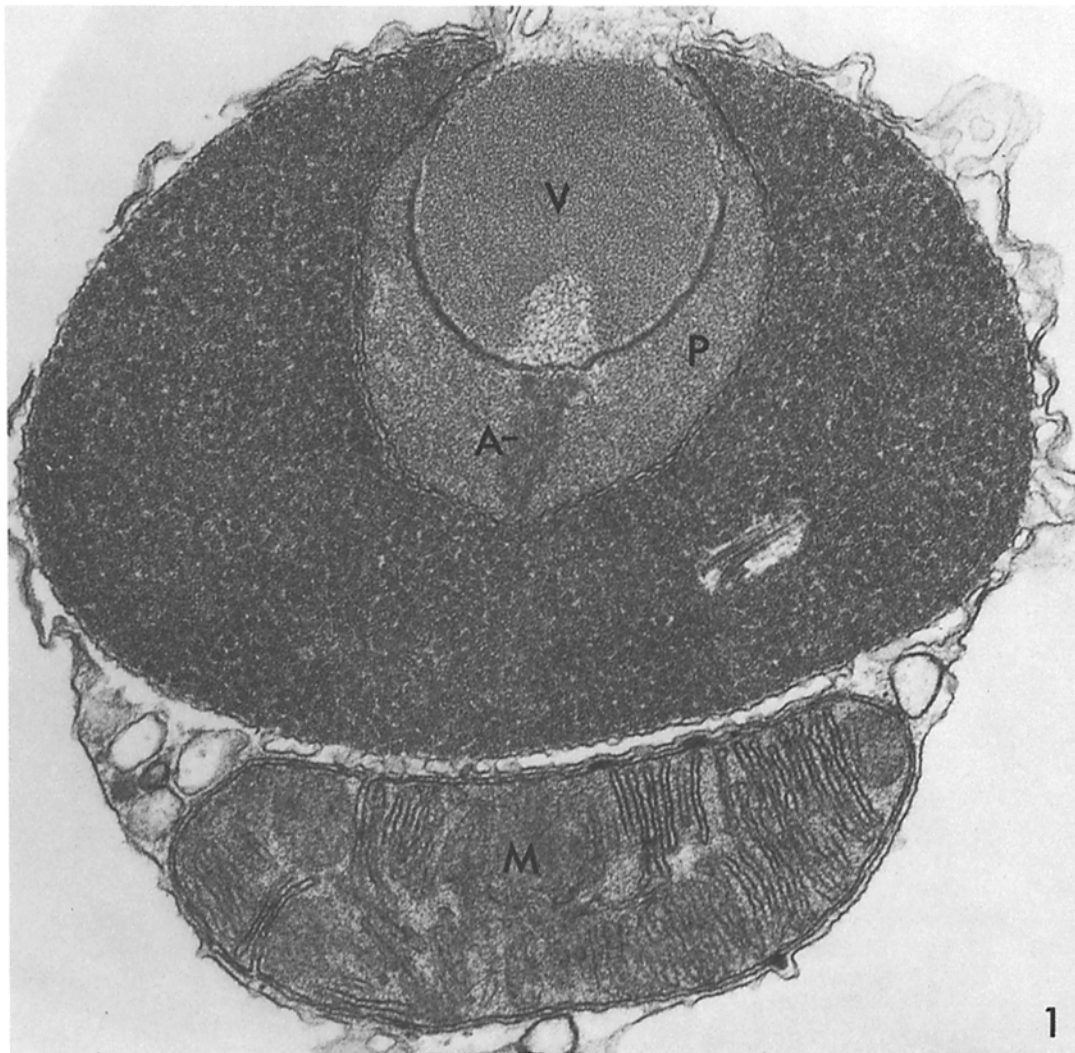


FIGURE 1 Thin section through a *Thyone* sperm which was suspended in 0.5 M NaCl containing 20 mM imidazole buffer at pH 7.8 and then fixed by the addition of glutaraldehyde to this suspension. Within an indentation in the nucleus (*N*) is the acrosomal vacuole (*V*) and the amorphous profilactin (*P*). In the center of this material is the actomere (*A*) which has a hint of filamentous material. Basal to the nucleus is the mitochondrion (*M*). The basal half of the acrosomal vacuole membrane appears thicker than the apical half.  $\times 70,000$ .

The basal half of the membrane limiting the acrosomal vacuole appears thicker than the apical half, due to some material attached to the inner or vacuolar surface of the membrane.

The structure of the actomere is revealed with greater clarity after the surrounding profilactin is removed. This is accomplished by extracting whole sperm with detergent at pH 8.0 (Figs. 2 and 3) or by treating sperm in seawater with X537A at pH 7.8 (Figs. 4 and 5). The former procedure solubilizes the profilactin (18); the latter induces the polymerization of the actin which is located in the profilactin (23). The fine structure of the actomere from sperm treated in both ways is identical. In longitudinal section (Figs. 2 and 4) the actomere appears as an electron-dense structure (0.1  $\mu\text{m}$  by 0.25  $\mu\text{m}$ ) composed of a parallel array of linear elements (filaments). At its widest dimension, one can count 6–7 filaments (Fig. 2). In transverse section (Figs. 3 and 5) the actomere consists of dots, each approx. 75  $\text{\AA}$  in diameter. These dots are the filaments cut in transverse section. The dots tend to be in rows indicating some organization although the order is not regular. There is some material around the filaments that makes it impossible to obtain an exact idea of the number, but, in examining many plates like Figs. 3 and 5, I have counted 20–25 filaments per actomere. The anterior end of the actomere consists of a dense plate which is particularly prominent in ionophore-treated sperm. (Fig. 4).

#### *Polymerization of Actin in Association with the Actomere*

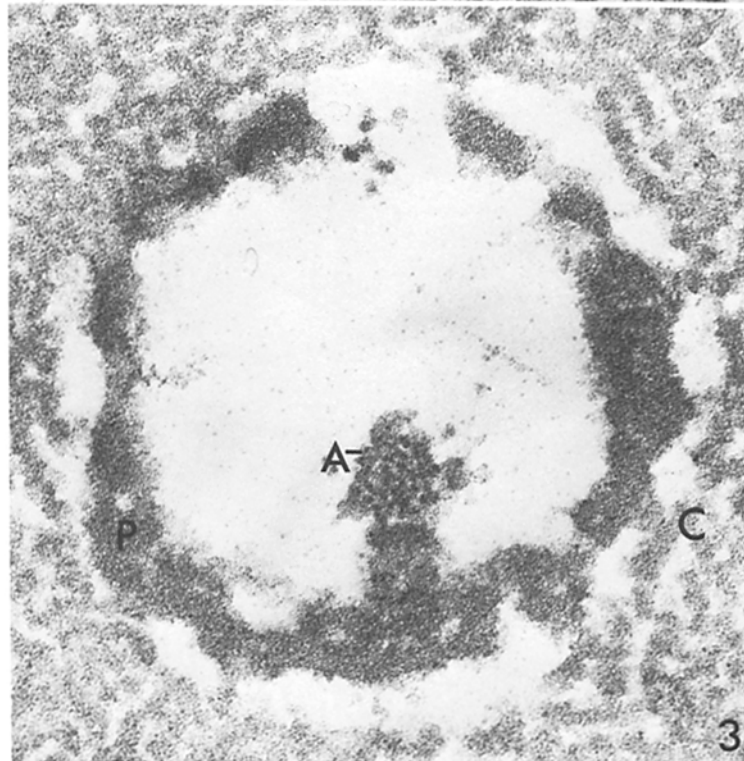
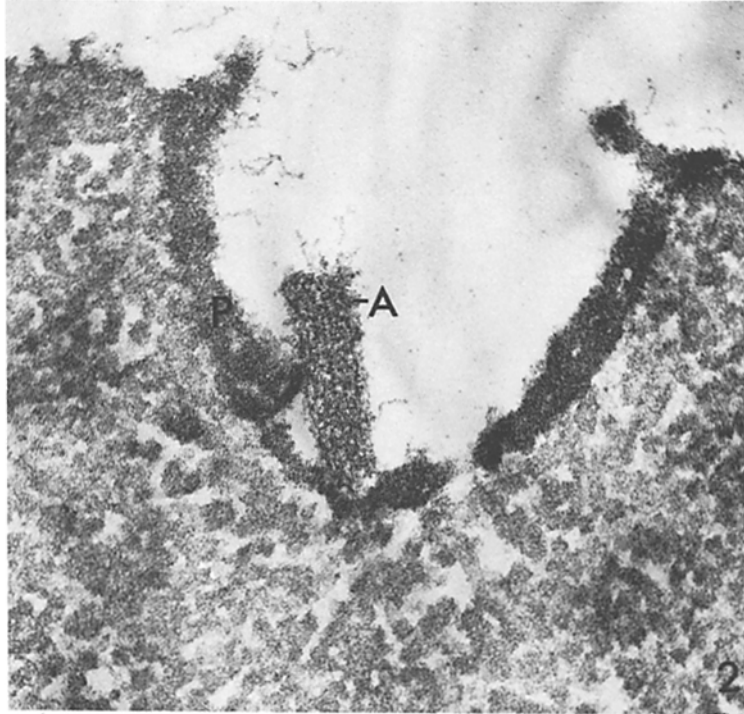
**DETERGENT-TREATED SPERM:** As mentioned in the introductory paragraph, when actin was encouraged to polymerize in vitro from the profilactin cups by adding ATP,  $\text{CaCl}_2$ , and  $\text{MgCl}_2$  to detergent-treated sperm, the actin polymerized only from the actomere (see Figs. 14 and 15 of reference 18). I repeated these observations under different conditions. *Thyone* sperm were demembrated with 0.5% Triton X-100 in 10 mM phosphate buffer and 1 mM  $\text{MgCl}_2$  at pH 6.4. The chromatin with its attached cup of profilactin was then incubated in a solution that should stimulate the polymerization of actin: 60 mM KCl, 1 mM Mg-ATP, 1 mM  $\text{MgCl}_2$  in 30 mM phosphate buffer at pH 7.2, at 22°C for 15 min. Under these conditions, the bulk of the profilactin disappears, leaving only the actomere (Fig. 6). Extending from the apical end of the actomere are some filaments. Similar results were obtained under

conditions in which demembrated sperm were incubated in 1 mM ATP, 1 mM  $\text{MgCl}_2$ , 1 mM  $\text{CaCl}_2$  in 30 mM Tris buffer at pH 7.5 for 10 min at 22°C (Fig. 7). The actin that did polymerize had polymerized from the actomere.

**ADDITION OF X537A TO THYONE SPERM IN SEAWATER AT PH 7.0:** In the preceding paper (23), we demonstrated that ionophore-induced polymerization of actin is dependent upon the external pH. If the pH of the medium is 8.0 (the pH of seawater), rapid polymerization occurs, but the filaments are randomly oriented (Fig. 4). At pH 6.5 (the presumed intracellular pH; reference 4), however, no polymerization of the actin occurs; at pH 7.0, only a partial polymerization of the actin takes place. From these experiments, I suspected that if the ionophore carries  $\text{H}^+$  out of the sperm at a slow rate due to a small concentration differential of  $\text{H}^+$  inside and outside the sperm cell, then perhaps some of the natural controls over the polymerization of actin are exerted and an organized bundle of filaments might appear.

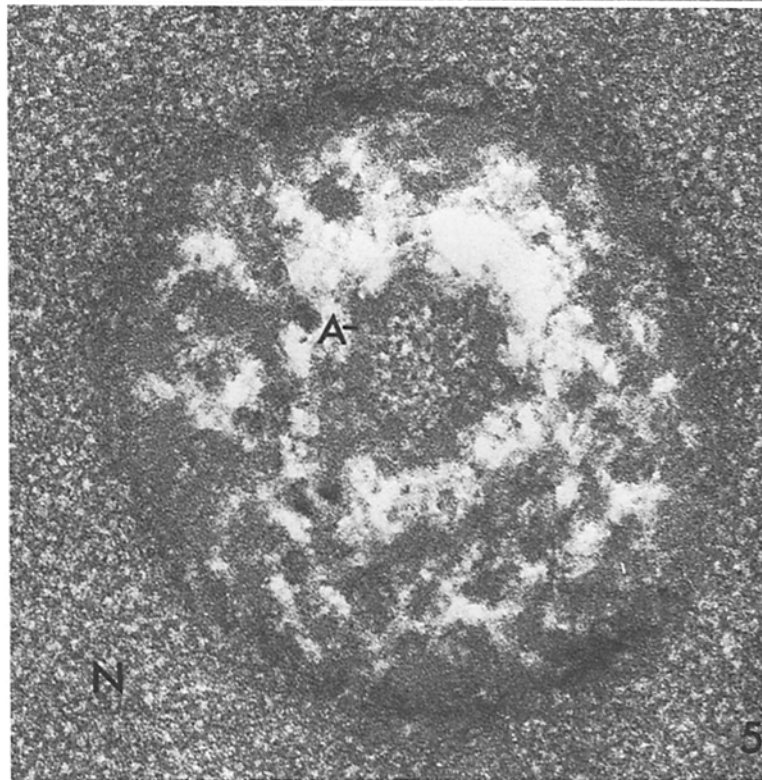
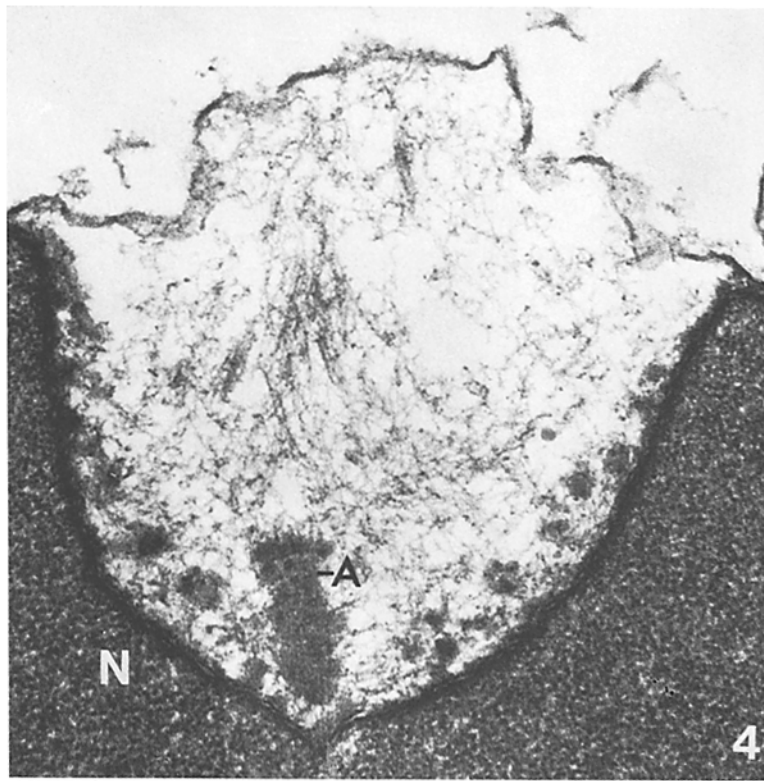
As was expected, most of the partial polymerization of actin that occurs when sperm are treated with X537A at pH 7.0 takes place near, or is associated with, the actomere and consists of a bundle of filaments extending anteriorly from it. What is extraordinary is that this bundle extends into, and in some cases all the way through, the acrosomal vacuole (Figs. 8–11)! The filament bundle does not penetrate the membrane limiting the acrosomal vacuole but rather pushes it ahead as one might push a finger into a soft rubber balloon. Thus, a tunnel is formed in the acrosomal vacuole, the walls being limited by the acrosomal vacuole membrane (Figs. 8 and 9). When the filament bundle pushes its way completely through the acrosomal vacuole, it produces a tiny process that extends out from the apical end of the sperm. In these cases, the projection containing filaments is covered by three membranes; the plasma membrane and two acrosomal vacuole membranes (Fig. 8). I have seen only a few instances in which the resultant process is covered by only two membranes indicating membrane fusion.

Of particular interest is the fact that the filament bundle that pushes its way into or through the acrosomal vacuole extends anteriorly from the actomere (Figs. 8, 10, and 11). Because of superposition artefacts created by the section thickness (a section can be minimally 200  $\text{\AA}$  thick, yet each actin filament is 50  $\text{\AA}$  in diameter) and the difficulty in resolving the substructure of the acto-



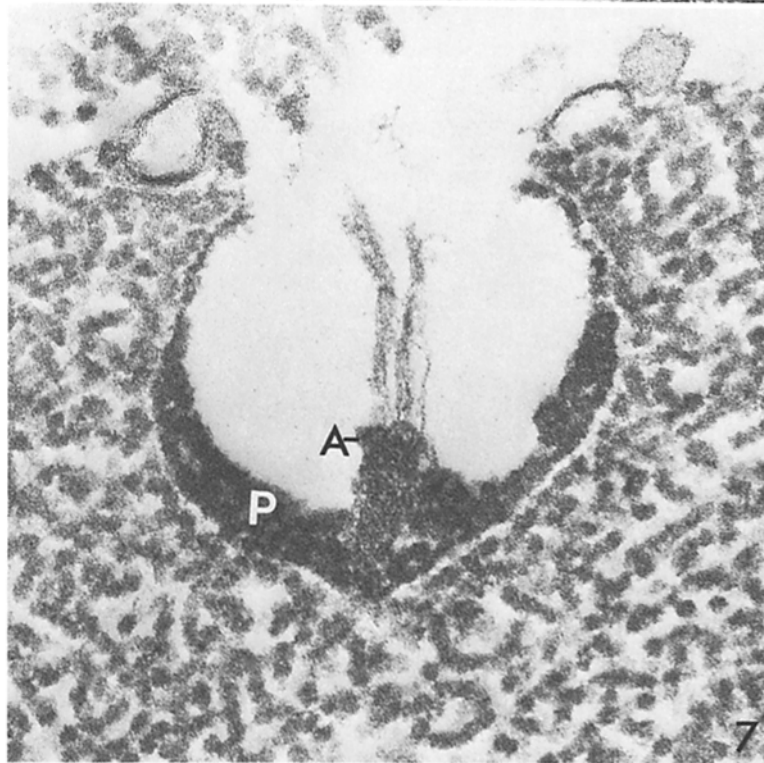
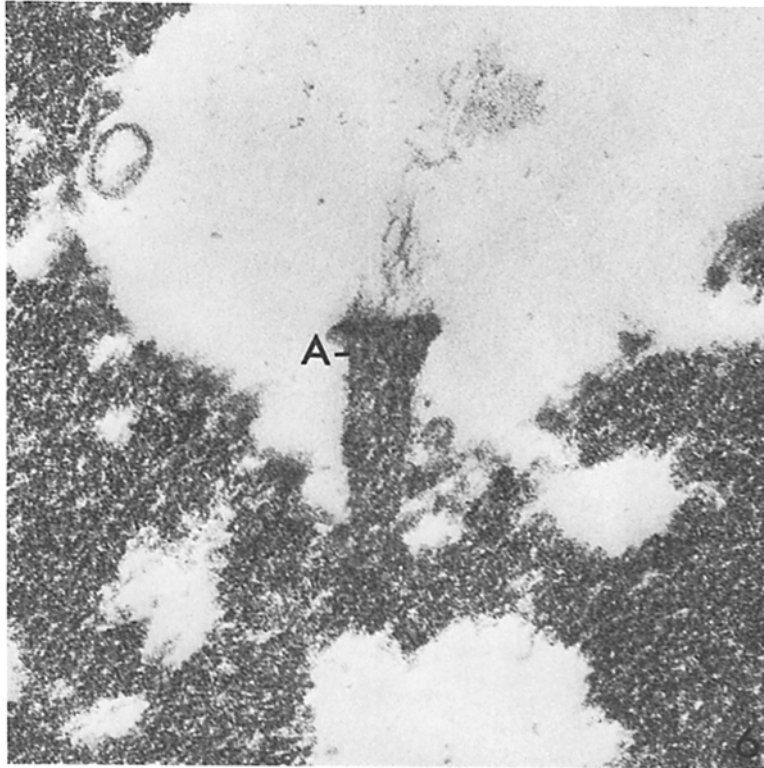
**FIGURE 2** The apical end of a *Thyone* spermatozoon which was demembrated with 1% Triton X-100 in 10 mM phosphate buffer at pH 6.4 containing 1 mM  $MgCl_2$ , then placed in 30 mM Tris-HCl and 3 mM  $MgCl_2$  at pH 8.0. Glutaraldehyde was then added to the medium. These conditions solubilize the cell membranes and much of the profilactin. Remaining in the indentation in the nucleus is a residual layer of profilactin (*P*), and in the center is the actomere (*A*). The actomere is composed of a parallel array of filaments.  $\times 96,000$ .

**FIGURE 3** Transverse section through an actomere (*A*). The sperm was treated as in Fig. 2. The residuum of the cup of profilactin (*P*) and the chromatin (*C*) are indicated. Note that the actomere consists of a series of dense dots which are filaments in transverse section.  $\times 135,000$ .



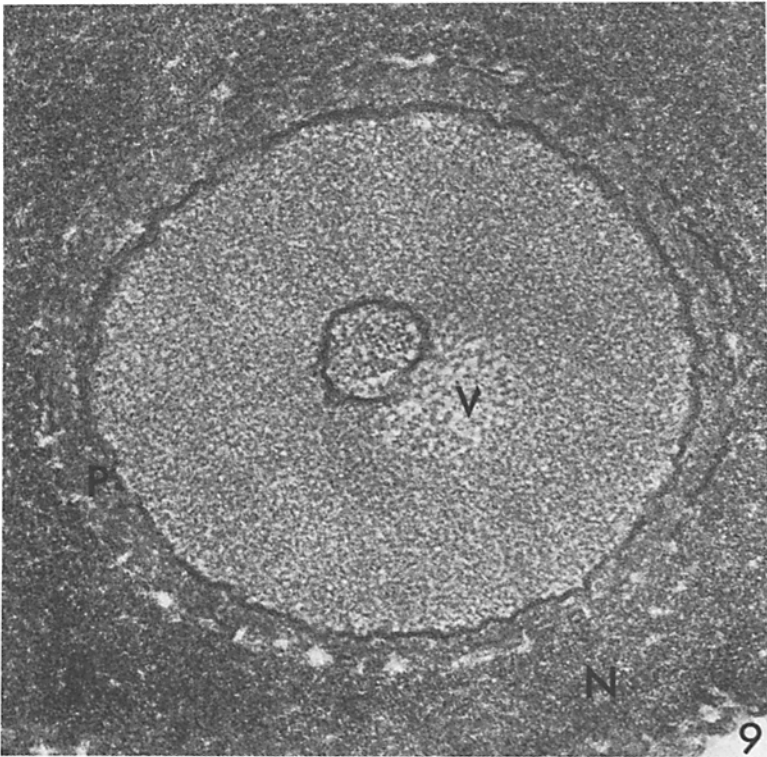
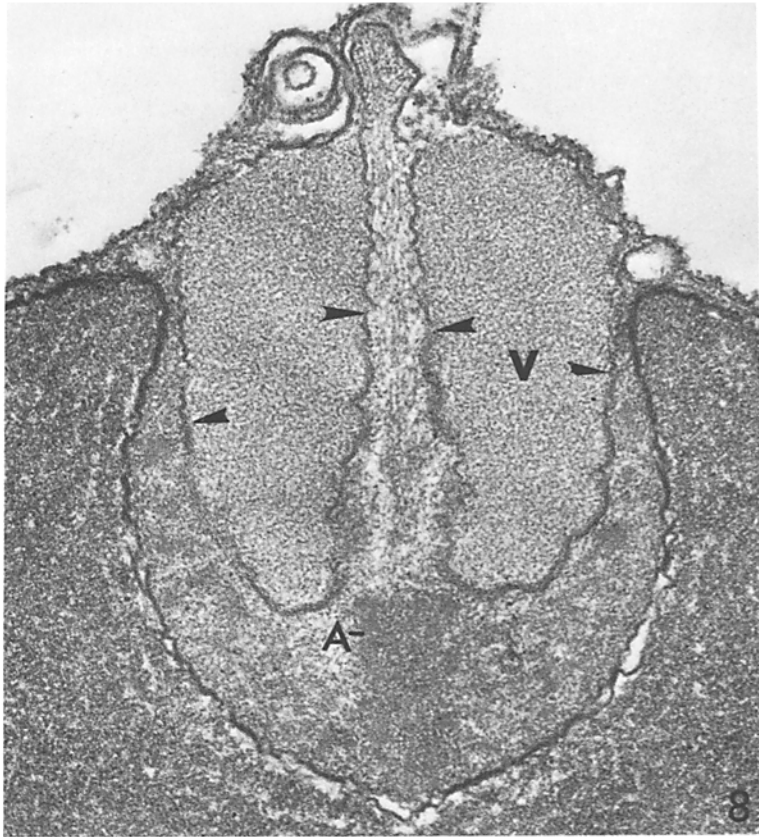
**FIGURE 4** Thin section through the apical end of a *Thyone* spermatozoon that was fixed 1.5 min after the ionophore X537A ( $10 \mu\text{l/ml}$  of a 1-mg/ml solution of X537A in ethanol) was added to the seawater (at pH 8.0) in which the sperm were suspended. The acrosomal vacuole has fused with the cell surface, and the profilactin has been converted into filaments. Of particular interest is the longitudinal section through the actomere (A). It is composed of filaments embedded in a dense matrix. The apical ends of the filaments insert into a dense plaque. A portion of the nucleus (N) is visible.  $\times 84,000$ .

**FIGURE 5** Transverse section through an actomere (A) from a spermatozoon treated as in Fig. 4. A portion of the nucleus (N) is visible. The actomere is composed of dense dots which are the filaments seen in longitudinal sections.  $\times 150,000$ .



**FIGURE 6** *Thyone* sperm were demembrated in 0.5% Triton X-100 in 10 mM phosphate buffer containing 1 mM  $MgCl_2$ , washed, and then placed in 30 mM Tris, 60 mM KCl, 1 mM Mg-ATP, and 1 mM  $MgCl_2$  at pH 7.2 at 22°C. Glutaraldehyde was added 15 min later. Under these conditions, the bulk of the profilactin disappears. The actomere (A) remains, and extending from its apical surface are some filaments.  $\times 150,000$ .

**FIGURE 7** *Thyone* sperm were demembrated as in Fig. 6, then placed in 1 mM ATP, 2mM  $MgCl_2$ , 1 mM  $CaCl_2$ , and 30 mM Tris buffer at pH 7.5 at 22°C. Glutaraldehyde was added 10 min later. Filaments can be seen extending from the actomere (A). Much of the profilactin remains.  $\times 93,000$ .





mere due to residual profilactin, I cannot demonstrate that the filaments in the bundle are direct extensions from the thicker filaments in the actomere, but this is a reasonable assumption based upon images such as those illustrated in Figs. 11 and 12 and based upon the in vitro polymerization results (Fig. 6). It is possible that the filaments in the bundle are not, in fact, continuous with the thicker filaments in the actomere, but rather extend from the dense material that is present on the apical surface of the actomere.

In the second paper in this series (17), I demonstrated that the membrane surrounding the acrosomal vacuole is differentiated into an apical half and a basal half, the basal half appearing thicker due to material that adheres to the vacuolar side of the membrane (Fig. 1). Careful examination of the acrosomal vacuole membrane lining the tunnel immediately peripheral to the filament bundle reveals that, whereas the membrane on the basal half of the tunnel is thicker, the membrane at the apical end resembles a "normal" unit membrane (see arrows, Fig. 8). This indicates that the production of new membrane may occur at the tip of the tunnel.

#### *Evidence for Lateral Association of the Filaments*

I have stated that the actomere is composed of 20–25 filaments. However, in transverse sections through the filament bundle in the center of the acrosomal vacuole (Fig. 9) or through the short process that extends out from the far side of the acrosomal vacuole (Fig. 10), more filaments can be seen. Furthermore, in transverse sections through the base of the acrosomal process of sperm induced to undergo a normal acrosomal reaction, as when a sperm meets an egg, or as when the sperm is treated with ammonia, there can be as many as 140 filaments (22). Thus, the

number of filaments in the actomere does not correlate accurately with the number of filaments in a process. This discrepancy can be understood by examining cases such as depicted in Fig. 13 in which the acrosomal vacuole has fused with the cell surface. Besides the filaments which extend from the actomere, a second population of filaments extends from the profilactin around the actomere. Some of these are randomly oriented (Fig. 11); others run parallel to the bundle of filaments extending from the actomere. Images such as Fig. 13 are consistent with the idea that, whereas the actomere organizes (perhaps by nucleation) the assembly of actin from the actomere, other filaments can be spontaneously nucleated and will tend to align themselves relative to an existing filament bundle. Cross-linking, then, presumably occurs in order to maintain the filaments in a bundle.

#### DISCUSSION

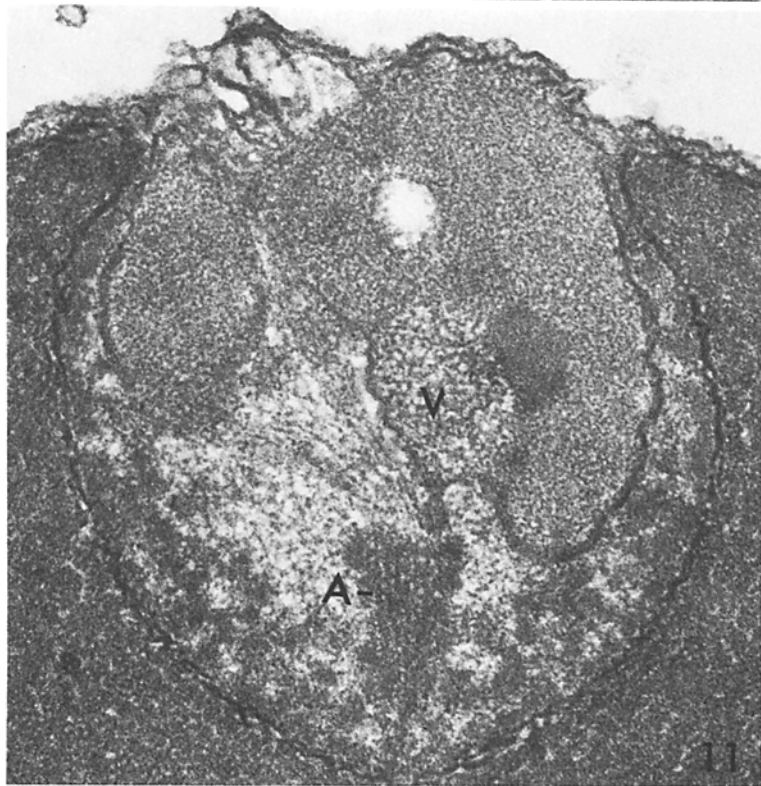
In this report, I have described the existence and morphology of a structure which I call the actomere. When the actin in the periacrosomal region is induced to assemble (both in vivo and in vitro), the actin filaments appear to form in association with the apical surface of the actomere. In fact, when sperm are induced to undergo the acrosomal reaction by an ionophore at pH 7.0, conditions under which most of the actin does not polymerize, the bulk of the filaments that do form extend anteriorly from the actomere with sufficient force to push their way through the acrosomal vacuole. Thus, the actomere gives direction to the growth and elongation of the actin filaments, a necessary step in the formation of a slender process whose axial ratio is generally 1,000:1 (90  $\mu\text{m}$  in length, yet seldom exceeding 0.1  $\mu\text{m}$  in diameter).

The most likely method by which the actomere would give direction to the filaments is that this

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FIGURE 8 Thin section through the apical end of a *Thyone* sperm that was fixed 1.5 min after the addition of the ionophore X537A (10  $\mu\text{l/ml}$  from a stock of 1 mg/ml of X537A in ethanol) to the seawater around the sperm. The seawater was maintained at pH 7.0 with 1 mM imidazole buffer. Extending from the actomere (A) is a bundle of filaments that pushes its way through the acrosomal vacuole (V). The arrows indicate the extent of the dense material attached to the inner surface of the acrosomal vacuole membrane.  $\times 90,000$ .

FIGURE 9 Thin section through the acrosomal vacuole (V), the profilactin (P), and a portion of the nucleus (N) from a *Thyone* sperm treated as in Fig. 8. The plane of section in this figure is at right angles to that of Fig. 8. Of particular interest is the center of the acrosomal vacuole where a filament bundle similar to that seen in Fig. 8 is cut in transverse section. The filaments appear as small dots.  $\times 90,000$ .



structure acts as a nucleation (initiation) center. Such a hypothesis is strengthened by the fact that the polarity of the filaments would be defined, as, for example, is true of the brush border and certain sperm systems (16). The reviewers of this manuscript suggested an alternative mechanism; the actomere may function by causing an aggregation of existing actin filaments which attach to it; subsequently, elongation could proceed. To distinguish between these alternatives requires a rigorous definition of nucleation.

Nucleation, as contrasted to condensation polymerization, can be recognized by alterations in the fourth-order kinetics of actin polymerization. Clearly, this type of kinetic analysis cannot be carried out on this system. Despite this rather rigorous definition, most investigators would agree that nucleation is occurring if the filaments start to grow from a structure and gradually increase in length. For example, we would say that the basal body of a cilium or flagellum nucleates the polymerization of the ciliary or flagellar axoneme, although it has not been proved; it could be that the basal body microtubules associate first with a small aggregate of tubulin and that then growth proceeds by condensation polymerization. The first step, then, would not be true nucleation. In a similar fashion the actomere may not, strictly speaking, nucleate actin polymerization. Instead, short filaments or an aggregate of actin may attach to the actomere, and then condensation polymerization may proceed. The problem, then, becomes one of determining what is the minimum-sized unit that will combine with the actomere. If it is a monomer, then indeed nucleation is truly occurring. If a short actin filament forms and secondarily attaches to the actomere, then the actomere is not nucleating polymerization but is acting as an organizing body.

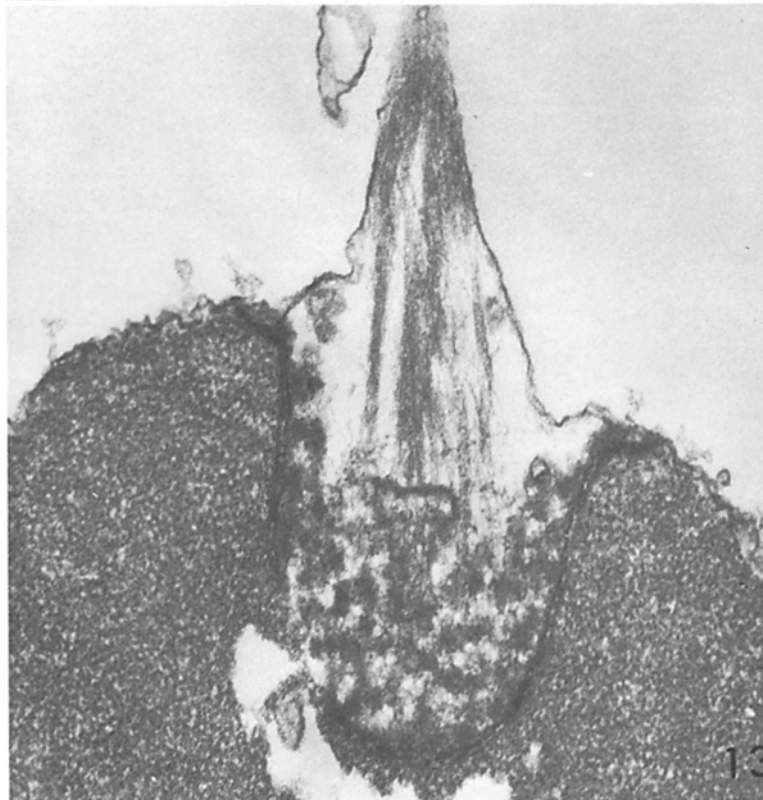
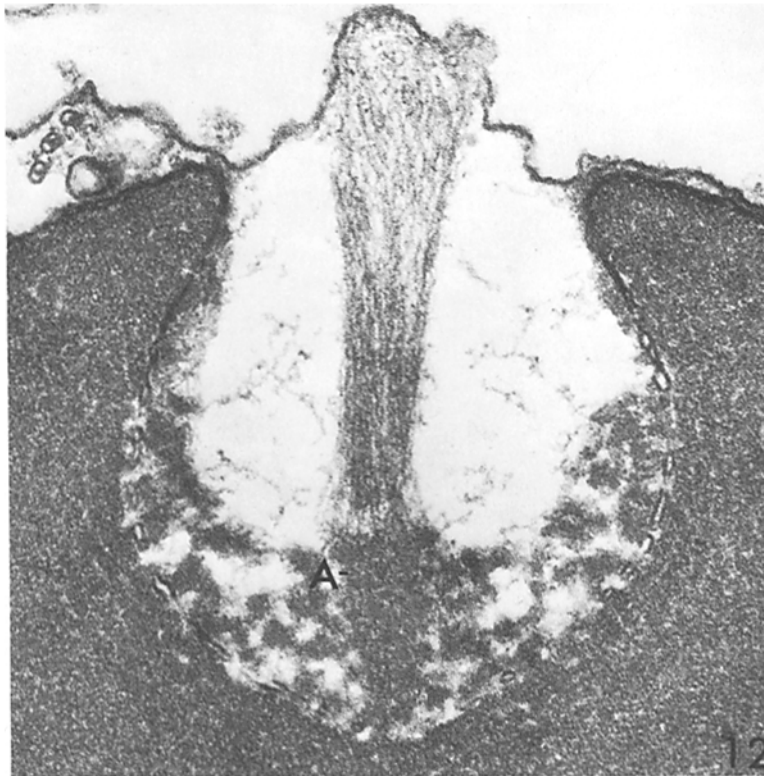
It has been documented by many investigators that in order for the actin to polymerize in vitro the actin monomers must be present in sufficient concentration. If they are below this concentration

(critical concentration), no polymerization can be induced. The rate of formation of filament nuclei is more concentration dependent than the rate of elongation of the filaments by the addition of actin to the ends. In vitro, raising the pH from 6.4 to 8.0 solubilizes the profilactin (18), releasing it from the other proteins present. Thus, if the spermatozoon controls the efflux of  $H^+$  so that the concentration of polymerizable actin or actin which has been released from the other proteins present is slightly above the critical concentration for polymerization, spontaneous nucleation will not be favored, but elongation of existing filaments will occur readily. However, if the concentration of the actin is well above the critical concentration, then spontaneous nucleation will also occur and filaments will form wherever there are monomers. I presume that the reason why we see actin filaments forming on the actomere only when sperm are treated with the ionophore at pH 7.0 is that at this pH little actin is freed from the other proteins, and thus the concentration of free actin is only slightly higher than the critical concentration for polymerization. At pH 8.0, on the other hand, most of the actin would be released from its binding proteins, allowing spontaneous nucleation to occur; then, actin filaments could form anywhere in the cell. I would predict, therefore, that controls over the concentration of free actin, i.e., by regulating the rate of  $H^+$  efflux, might be the way in which the sperm cell normally elicits the formation of a needlelike cell extension rather than a mushroomlike growth. The efflux of  $H^+$  is probably sufficiently rapid to maintain the concentration of polymerizable actin at a slightly higher level than just above the critical concentration, because there are more filaments (up to 140) in a fully formed acrosomal process than there are initially in the actomere (20-25). An examination of Fig. 13 indicates that the filaments attached to, and, in my model, nucleated from, the actomere are probably in the center of the acrosomal process, the filaments at the periphery presumably forming as the result of spontaneous nucleation.

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FIGURE 10 Thin section through the apical end of a *Thyone* sperm treated as in Fig. 8. Extending from the actomere (A) is a bundle of filaments that extend through the acrosomal vacuole (V). The filament bundle is cut in nearly transverse section (indicated by the arrow) at the most anterior end of the acrosomal vacuole.  $\times 93,000$ .

FIGURE 11 Thin section through the apical end of a *Thyone* sperm treated with X537A in seawater at pH 7.0 as in Fig. 8. Filaments can be seen extending from the actomere (A). The acrosomal vacuole (V) has a very asymmetric shape.  $\times 100,000$ .



The formation of a thin process rather than a mushroom may be the result, then, not only of controlled nucleation but of the organization of the filaments into a bundle presumably by cross-linking, which seems to be a reasonable assumption based upon the final length (90  $\mu\text{m}$ ) and breadth (0.05–0.1  $\mu\text{m}$ ) of the acrosomal process. The actomere may also act as the site for side-by-side aggregation.

The concept of an organelle that might nucleate the assembly of actin filaments in relation to changes in the internal ion composition is an exciting one, but one which poses more questions for further investigation. Immediately, one would like to know whether the polymerization of actin *in vivo* is associated with the splitting of ATP, since *in vitro* each monomer splits ATP when it polymerizes to the polymer. This is not an absolute requirement as demonstrated by Kasai et al. (7), but, in general, ATP will be cleaved if present. This means that  $\text{H}^+$  would be generated upon polymerization, which in turn would lower the intracellular pH enabling less actin to be released from the profilactin complex at least until more  $\text{H}^+$  either is pumped out of the cell, diffuses out of the cell due to a depolarization of the membrane, is buffered away, or any combination of the above. The production of  $\text{H}^+$  by ATP splitting may be an important factor in controlling the critical concentration of unbound actin. Perhaps the cell has several mechanisms by which it may control the level of unbound actin.

One wonders also about the ubiquity of the actomere. Will it be found in other sperm or in other cells? Very little information pertaining to this question is available. A more relevant question may be: What is an actomere? As demonstrated here, an actomere is a collection of filaments embedded in a dense matrix. I suspect that this dense matrix may turn out to be the material that is generally associated with the assembly of actin filaments. My supposition here is based upon two facts: (a) During the assembly of actin filaments in microvilli, the actin is assembled from a dense substance, not a dense substance with fila-

ments embedded in it (19). This substance may be composed in part of  $\alpha$ -actinin (13). (b) In other sperm such as those of the starfish, *Pisaster*, many of the filaments in the profilactin arise from an organelle homologous to the actomere. This structure, however, is composed of a dense material with no preformed filaments embedded within it.

There is an interesting parallel here between the assembly of actin filaments and the assembly of microtubules that is perhaps pertinent to our understanding of how structural proteins are organized in cells. Microtubules are also nonrandomly distributed in cells; this organization is brought about in part by the nucleation of assembly of microtubules from small densities in the cytoplasm of the cells. These densities have been called various names, such as nucleating centers (20) or MTOC (12). Yet, as seems to be the case of the actomere in *Thyone* sperm, there is an organelle, the basal body, or centriole, which also seems to nucleate microtubule growth: in this case, the growth of the flagellar or ciliary axoneme. The basal body may be, in reality, just an extension of the dense nucleating sites or MTOC's, the only difference being that there are microtubules embedded in the dense material. Thus, in both cases, tubulin and actin, there is evidence that the nonrandom distribution of at least some of the filaments or tubules is determined by nucleation from small dense foci. I do not mean to imply that this dense substance is of the same chemical composition in both cases, but only to suggest that in both systems there can exist a morphologically distinct organelle, such as the centriole or actomere, as well as morphologically indistinct sites such as the MTOC's for tubulin polymerization, or the dense bodies, Z disk, and dense material ( $\alpha$ -actinin) for actin polymerization.

It is my great pleasure to acknowledge stimulating discussion with my colleagues Dan Kiehart, Ray Stephens, Sally Zigmond, and Tom Pollard during the course of these experiments. I would also like to thank the reviewers of this paper for suggesting an alternative explanation for the data presented. I would particularly

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FIGURE 12 Thin section through the apical end of a *Thyone* sperm treated with X537A in seawater at pH 7.0 as in Fig. 8. The acrosomal vacuole has fused with the cell surface. Filaments can be seen extending from the actomere.  $\times 90,000$ .

FIGURE 13 Thin section through the apical end of a *Thyone* sperm treated as in Fig. 8. The acrosomal vacuole has fused with the cell surface. Filaments not only extend from the actomere but also from the profilactin lateral to the actomere.  $\times 95,000$ .

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