Actin from *Thyone* Sperm Assembles on Only One End of an Actin Filament: A Behavior Regulated by Profilin

LEWIS G. TILNEY,* EDWARD M. BONDER,*‡ LYNNE M. COLUCCIO,* and MARK S. MOOSEKER‡
*Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and ‡Biology Department, Yale University, New Haven, Connecticut 06520

ABSTRACT *Thyone* sperm were demembranated with Triton X-100 and, after washing, extracted with 30 mM Tris at pH 8.0 and 1 mM $MgCl_2$. After the insoluble contaminants were removed by centrifugation, the sperm extract was warmed to 22°C. Actin filaments rapidly assembled and aggregated into bundles when KCl was added to the extract. When we added preformed actin filaments, i.e., the acrosomal filament bundles of *Limulus* sperm, to the extract, the actin monomers rapidly assembled on these filaments. What was unexpected was that assembly took place on only one end of the bundle—the end corresponding to the preferred end for monomer addition. We showed that the absence of growth on the nonpreferred end was not due to the presence of a capper because exogenously added actin readily assembled on both ends. We also analyzed the sperm extract by SDS gel electrophoresis. Two major proteins were present in a 1:1 molar ratio: actin and a 12,500-dalton protein whose apparent isoelectric point was 8.4. The 12,500-dalton protein was purified by DEAE chromatography. We concluded that it is profilin because of its size, isoelectric point, molar ratio to actin, inability to bind to DEAE, and its effect on actin assembly. When profilin was added to actin in the presence of *Limulus* bundles, addition of monomers on the nonpreferred end of the bundle was inhibited, even though actin by itself assembled on both ends. Using the *Limulus* bundles as nuclei, we determined the critical concentration for assembly off each end of the filament and estimated the K_d for the profilin-actin complex ($\sim 10\ \mu M$). We present a model to explain how profilin may regulate the extension of the *Thyone* acrosomal process in vivo: The profilin-actin complex can add to only the preferred end of the filament bundle. Once the actin monomer is bound to the filament, the profilin is released, and is available to bind to additional actin monomers. This mechanism accounts for the rapid rate of filament elongation in the acrosomal process in vivo.

In the past 15 years an enormous amount of information has been accumulated about actin in nonmuscle cells. It now seems clear that the antics of this ubiquitous protein appear to be regulated by a seemingly endless number of proteins (5, 10). Although a great deal is known about the behavior of many of these proteins in vitro, it is apparent that their functions in vivo are not well understood. This is not surprising as individual cells, to say nothing of whole organs, are involved in hundreds of different processes, many of which go on simultaneously. For example, a cell such as an amoeba can be simultaneously involved in pseudopodial movement, phagocytosis, contraction of the contractile vacuole, mitosis, cytokinesis, protein synthesis, movement of the newly synthesized material to the Golgi apparatus, lysosomal discharge and/or fusion with digestive vacuoles, streaming, etc. Thus, if one isolates a protein, e.g., profilin, from an amoeba or perhaps a spleen (a complex organ), it is difficult to relate what this protein can do in vitro with the multitude of movements the cell is actually doing in vivo.

Another approach to understanding the function of actin in vivo is to select a cell that is so highly specialized that it carries out a more limited repertory of functions. Then, by studying what such a specialized cell is doing, we are in a better position to relate these functions to the biochemistry of the proteins involved. There are two obvious difficulties with this approach. First, because the systems are extremely

THE JOURNAL OF CELL BIOLOGY · VOLUME 97 JULY 1983 112–124
© The Rockefeller University Press · 0021-9525/83/07/0112/13 $1.00

specialized, there is always the concern that what we are studying is not a general property of all cells of the body but only an "oddball" function carried out by a few of its cells. Second, one is generally limited by the amount of available material because these specialized cells are few in number, the organisms are hard to obtain in large numbers, or because of seasonal constraints.

We selected the acrosomal reaction of the sperm of an echinoderm, *Thyone*, as our experimental system. During the acrosomal reaction there is an explosive polymerization of actin that is responsible for the extension of the acrosomal process. Information is now available on what ions trigger the actin assembly in vivo (19, 27), how the filaments are nucleated and grow in one direction (23, 25), what the kinetics of elongation of the process are and thus the rate of actin assembly (24), what the initial steps in the reaction are (9, 27), and to which end of the elongating filaments the monomeric actin probably adds in vivo (24). This last point is interesting since recent evidence on the kinetics of elongation of the acrosomal process (24) is consistent with a model in which the addition of actin monomers to the elongating filaments occurs on the end located at the tip of the acrosomal process. Such a hypothesis coincides with the polarity of the actin filaments in the process where the preferred end for monomer addition is the tip of the process (26, 31).

In this paper we report on some experiments in which we examined an extract from *Thyone* sperm that contained actin, along with an actin-binding protein. The *Thyone* actin was then induced to assemble in vitro. The assembly was nucleated by adding segments of the preformed bundle of actin filaments isolated from *Limulus* sperm (26). The *Limulus* bundle is particularly advantageous as a nucleating body because not only is it extremely stable but it tapers so that at a glance one can identify which end of the bundle is preferred for monomer addition—it is the thinner end of the bundle (26). We discovered that the assembly of monomers from the *Thyone* extract occurs on only one end of the *Limulus* bundles, the preferred end. Evidence is presented that this unidirectional addition of monomers is governed by a second protein, profilin. These observations extend the earlier in vivo observations and illustrate how at least one of the actin regulatory proteins may be behaving in vivo.

MATERIALS AND METHODS

Obtaining Organisms and Sperm: *Thyone briareus* and *Limulus polyphemus* were collected by the Department of Marine Resources at the Marine Biological Laboratory (Woods Hole, MA) and kept in aquaria with running sea water, in Instant Ocean Aquaria (Instant Ocean Aquarium Systems, Eastlake, OH) at the University of Pennsylvania, or in recirculating sea water at Yale University. Sperm were obtained from *Limulus* as described by Tilney (21). *Thyone* sperm were released from the testis by mincing it in filtered sea water. The sperm suspension was then filtered through cheesecloth and concentrated by centrifugation (1,000 *g* for 5 min).

Preparation of Thyone Extract for Assembly Studies: *Thyone* sperm were suspended in sea water and diluted until the optical density (OD; at 350 nm) of the suspension measured 1.7 "OD units." 200 ml of this suspension was used for each experiment. The sperm in the suspension were pelleted (1,000 × *g* for 5 min) and then extracted with 2 ml of a solution containing 1% Triton X-100, 10 mM PIPES, and 1 mM $MgCl_2$ at pH 6.3 with 4% Aprotinin (Sigma Chemical Co., St. Louis, MO) present to inhibit possible proteolysis. The demembranated sperm were pelleted (750 *g* for 5 min) in 15-ml Corex tubes in a Sorvall RC2B centrifuge with an SS34 head (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT). The extraction and all subsequent steps in the isolation procedure were carried out at 0–4°C. The supernate was discarded and the pellet washed with 2 ml of the PIPES-Magnesium-Aprotinin solution and recentrifuged (750 *g* for 5 min). The pellet was resuspended in 2 ml of 50 mM phosphate buffer at pH 6.3 containing 1 mM $MgCl_2$ and 1% Aprotinin (a procedure that removed most of the remaining flagella), and the suspension was recentrifuged (750 *g* for 5 min). The supernate, containing most of the flagellar axonemes, was removed and the pellet extracted for 20 min with 2 ml of 30 mM Tris, 1 mM $MgCl_2$, and 0.1 mM ATP at pH 8.0 containing 1% Aprotinin. The solution was then centrifuged (10,000 *g* for 10 min) and the supernate was removed and saved. We will refer to the supernate as the sperm extract. At each stage in the processing of the *Thyone* sperm a small sample was examined with oil immersion (100-× objective) in a Zeiss phase contrast microscope to ascertain whether or not the profilactin cup was present (22), and at the last stage, to determine the degree of extraction of this cup.

Gel Electrophoresis: SDS PAGE was carried out with 4–16% mini slab gels according to the method of Matsudaira and Burgess (15). The molecular weight of sperm profilin was determined by running molecular weight standards on adjacent lanes to the sperm profilin using 12.5% SDS PAGE slab gels according to the method of Laemmli (11). In trying to determine the optimum method for extracting actin from sperm it was necessary to run not only the supernate, but also the pellet which, in sperm, is mostly DNA. In SDS the DNA uncoils and produces a horrendous gel which, if run on SDS PAGE, smears the proteins running in it. We found that by using a stacking gel, most of the DNA is held there, which allows the proteins present to migrate normally. The gels must be loaded by squirting the boiling hot samples into the appropriate gel slot. If the samples are allowed to cool, they solidify. Gels were stained with Coomassie Brilliant Blue. Sperm extracts were also subjected to nonequilibrium pH gradient gel electrophoresis (NEPHGE) according to the method of O'Farrell et al. (16). NEPHGE gels were stained according to the method of Weber and Osborn (30).

Isolation of the False Discharge of Limulus Sperm: *Limulus* sperm were extracted with 1% Triton X-100 with 30 mM Tris and 3 mM $MgCl_2$ at pH 8.0, which removes the membranes and induces the filament bundle to transform into the false discharge state (21). The extract was then spun (750 *g* for 5 min) and the supernate was saved. The supernate was then centrifuged (10,000 *g*), pelleting both the false discharges and the flagellar axonemes. The pellet was extracted with 0.5% Sarkosyl (Ciba-Geigy Corp., Ardsley, NY) in 10 mM Tris at pH 7.5, which solubilizes the flagellar axonemes, leaving behind a purified preparation of false discharges (21). Prior to extraction with Sarkosyl, the preparation was washed with 10 mM Tris to remove Mg^{++} which, if not removed, precipitates the Sarkosyl. In some cases the Sarkosyl step was eliminated because axonemes are easy to distinguish from false discharges in the electron microscope.

Induction of Filament Assembly from Sperm Extracts: The *Thyone* actin present in the sperm extract was induced to polymerize by increasing the salt concentration in the extract to 2 mM $MgCl_2$, or 2 mM $MgCl_2$ and 60 mM KCl, and then incubated from 15 min to 2 h at room temperature. At various time points samples of the extract were negatively stained. The extracts were then centrifuged (100,000 *g* for 2 h at 4°C), and the pellets and supernates were processed for SDS PAGE. In some experiments the false discharges were also added to the sperm extract to nucleate filament assembly, and the pattern of assembly was assayed by negative staining and SDS PAGE.

Effect of Purified Profilin on Assembly on the Nonpreferred End: Concentrated solutions of KCl and $MgCl_2$ were added to the sperm extract to make it 60 mM KCl and 2 mM $MgCl_2$ and then, after incubation for 1 h on ice, the extract was centrifuged (100,000 *g* for 1 h). The supernate was loaded onto a DEAE column previously equilibrated with 60 mM KCl, 2 mM $MgCl_2$, 30 mM Tris at pH 8.0, and 0.2 mM dithiothreitol (DTT). Profilin was quantitatively removed from the column by elution with the above equilibration buffer. The purified profilin was then concentrated using dialysis against Aquacide II (Calbiochem-Behring Corp., La Jolla, CA) and dialyzed in 2 mM Tris at pH 8.0 containing 0.2 mM $CaCl_2$ and 0.5 mM DTT.

DEAE column-purified profilin (5–10 μM) was mixed with the false discharges and salt added such that the final salt concentration was 75 mM KCl, 2 mM $MgCl_2$, and 10 mM imidazole at pH 7.5. Gel-filtered G actin from skeletal muscle (13) was then added to a final concentration of 0.5 μM, incubated for 6 min, and negatively stained. In control preparations an equal volume of profilin dialysis buffer was substituted for the profilin. The false discharges were then examined for filament growth from both the preferred and nonpreferred ends of the filaments making up the false discharge.

Determination of Critical Concentration for Actin Assembly Off the Two Ends of the False Discharge: False discharges were suspended in 75 mM KCl, 2 mM $MgCl_2$, and 10 mM imidazole at pH 7.5 to which varying concentrations of gel-filtered G actin (0.1–1.5 μM) were added. In other experiments they were suspended in 60 mM KCl and 2 mM $MgCl_2$ in Tris at pH 8.0. These mixtures were then incubated for various time periods (see Results) and negatively stained. The false discharges were examined for filament growth at both ends of the bundle.

Electron Microscopy: Negative staining was done on copper grids coated with either parlodion or formvar and stabilized by a thin layer of carbon. Immediately prior to use, the grids were glow discharged to improve wetting. All samples were stained using 1% aqueous uranyl acetate. Grids were examined in a Philips 200 or 201 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ).

Other Methods: For all experiments skeletal muscle actin was prepared according to Spudich and Watt (20) and further purified by gel filtration following the method of MacLean-Fletcher and Pollard (13). Protein concentrations were determined according to Lowry et al. (12). Actin concentration was also determined using the extinction coefficient as determined by MacLean-Fletcher and Pollard (13).

RESULTS

To acquaint those readers unfamiliar with the formation of the acrosomal process in *Thyone* sperm, we have included a diagram (Fig. 1) of this magnificent event (9, 24) because an understanding of the biology is essential for subsequent discussion. Briefly, when the sperm makes contact with the jelly surrounding a *Thyone* egg, the acrosomal reaction is induced. The first detectable event seems to be the popping open of the acrosomal vacuole (9), followed by the fusion of the acrosomal vacuole membrane with the cell surface. This is followed by the assembly of actin, which was formerly sequestered in the profilactin region (22), onto the distal end of the actomere ([25], Figs. 1, *b–e*).

Extraction of the Actin from Thyone Sperm

Previously Tilney (22) demonstrated that if sperm are extracted with the detergent, Triton X-100, the membranes solubilize, but the profilactin remains intact, provided that the pH is maintained below pH 6.4; if the pH is raised to 8.0, the profilactin solubilizes.

An SDS gel of the pellets and supernates at each stage in the preparation of the sperm extract is depicted in Fig. 2 (S_4). In the final pH 8.0 extract (S_4) we found two major proteins, actin and a protein (labeled *P* in Fig. 2) whose apparent molecular weight is 12,500. Also present in the supernate were other minor components, the amount of which varies from preparation to preparation. There is often a band immediately below actin, which we feel is probably a proteolytic fragment caused by cleavage of the actin with proteases initially present in the acrosomal vacuole (22). It is interesting that almost all of the actin was extracted from the sperm by the high pH extraction procedure (compare P_4 with S_4), yet little was extracted prior to this (compare S_1–S_3 with S_4). At the same time the P-protein was missing in S_1–S_3 but was prominent in S_4. The four histone proteins (*H*) were incredibly strong in the pellets P_1–P_4, but absent in the supernate (S_1–S_4). Thus, our procedure preferentially extracted the actin and the P-protein from the sperm.

We should mention that in the final extract we did not find the high molecular weight bands (spectrin-like) initially described by Tilney (22). This material was largely extracted in the first wash with Triton X-100 (S_1). The PIPES buffer seemed to extract these proteins, unlike the phosphate buffered-Triton extraction solution used in earlier studies (22). Furthermore, the initial quantity of sperm that was processed and the volume of each solution in which the sperm were suspended had to be carefully controlled at each stage. If they were not, only a small amount of the cup would be extracted or the chromatin in the sperm head would form a DNA gel. The chromatin begins to uncoil in 1 mM $MgCl_2$, but because we started with a pellet of sperm suspended in sea water, at least for the first step or two, enough residual Mg^{++} and Ca^{++} was carried along to maintain the compactness of the chromatin. The chromatin was just beginning to uncoil at the final extraction step. If the concentration of $MgCl_2$ is higher at these steps, the yield of actin at the final stage is much less. Thus, by our procedure we have reduced the $MgCl_2$ concentration to as low a value as possible without causing the chromatin to unwind.

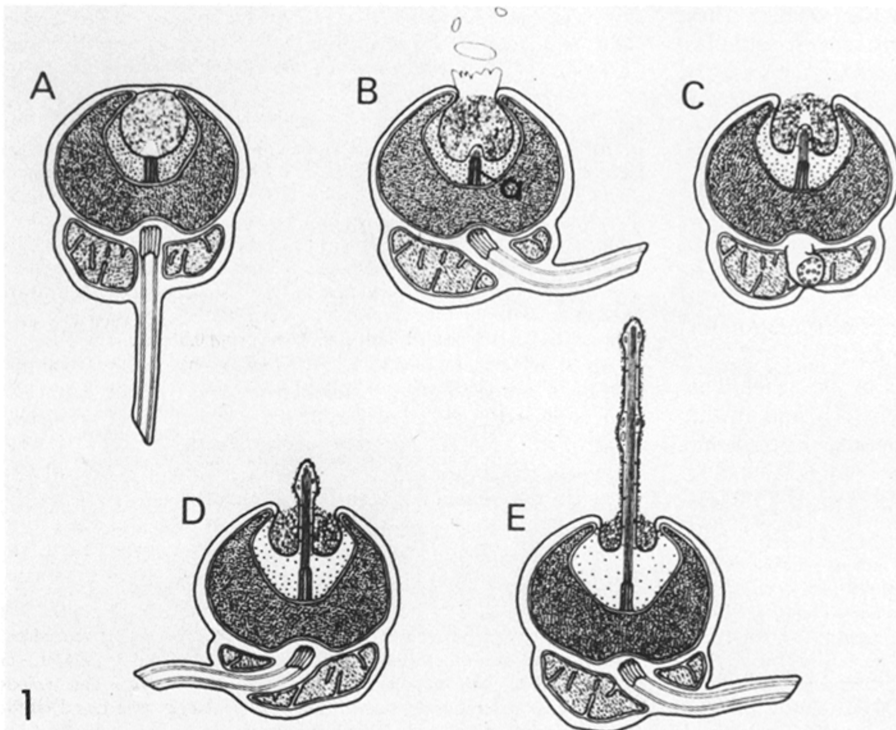


FIGURE 1 Schematic representation of the structural changes that occur in *Thyone* sperm undergoing the acrosomal reaction. (*A*) *Thyone* prior to induction. (*B*) As the membrane limiting the acrosomal vacuole is fusing with the plasma membrane, the actin begins to polymerize on the actomere (a). (*C–E*) Further stages in this reaction. Ultimately a process 90 μm long is produced from a sperm whose diameter is $\sim$2 μm. (Reproduced from the *Journal of Cell Biology*, 1981, 93, 812–819, by permission of The Rockefeller University Press.)

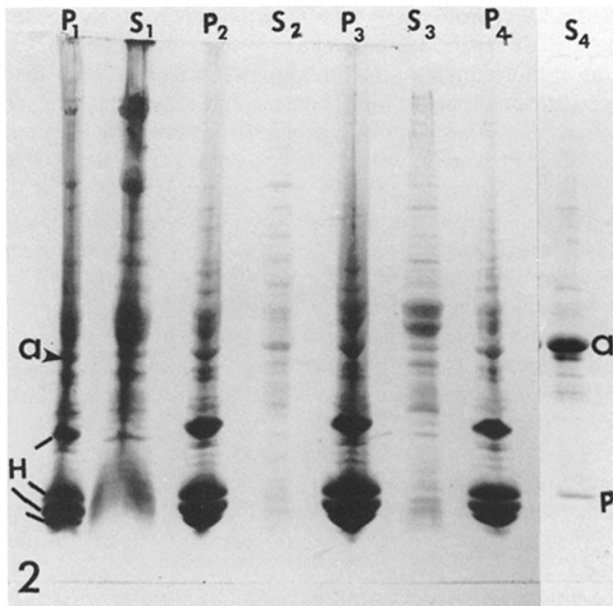


FIGURE 2 12.5% SDS PAGE slab gel of sperm extraction. *Thyone* sperm were first extracted with 1% Triton X-100 containing 10 mM PIPES, and 1 mM $MgCl_2$ at pH 6.3, and then pelleted. The pellet (P_1) contains many proteins, most prominent of which are the four histone bands (*H*). A band of the same electrophoretic mobility as actin (a) is visible. The supernate (S_1) also contains many bands, but note that the histone bands are absent and only a little bit of actin is present. The pellet was then washed with 10 mM PIPES containing 1 mM $MgCl_2$ at pH 6.3 and the insoluble material pelleted (P_2). As before, P_2 contains the four histone bands and a prominent actin-like band. The supernate (S_2) contains only traces of proteins. The pellet P_2 was then resuspended in 50 mM phosphate buffer at pH 6.3 containing 1 mM $MgCl_2$ and the preparation spun at 750 *g*. The resultant supernate (S_3) contains two prominent bands that are the tubulins since flagella are left in the supernate. Very little actin is present in this lane. In the pellet (P_3) again the most prominent bands are the histones with some tubulin and a prominent actin band. The pellet (P_3) was finally extracted with 1 mM $MgCl_2$ and 30 mM Tris at pH 8.0, centrifuged at 10,000 *g* for 10 min. In the pellet (P_4) we see the four histone bands, some tubulin, and a very small amount of the actin band; however, the supernate (S_4), referred to as the sperm extract, contains a very prominent actin band (*a*) and a low molecular weight substance labeled *P*.

Incubation of the Sperm Extract at Room Temperature Induced the Actin in the Extract to Assemble into Filaments

When the extract was incubated at room temperature for 15 min to 2 h and then spun (100,000 *g* for 2 h at 4°C), we found that at least 50% of the actin was in the pellet. The addition of KCl to 60 mM and/or $MgCl_2$ to 2 mM in the extract did not appreciably increase the amount of sedimentable actin. It is interesting that all the other bands, including the band labeled *P*, were present in the supernate. If ATP was present in the extraction buffer (E_2 in Fig. 3 *a*), the amount of actin in the pellet (compare P_2 with P_1 in Fig. 3 *a*) tended to be higher. The addition of assembly nuclei (*Limulus* false discharges) to the extract did not increase the amount of actin in the pellet nor did the addition of phalloidin at 1–10 μM.

We examined the sperm extract (note that the extraction medium consisted of 30 mM Tris, 1 mM $MgCl_2$, and 0.1 mM ATP at pH 8.0) by negative staining immediately before subjecting it to a high speed spin and found randomly oriented actin filaments (Fig. 4 *a*). Thus, the actin in the high speed pellet consisted, at least in part, of newly assembled actin filaments. We also examined a sperm extract containing 60 mM KCl in the Tris-Mg-ATP buffer. After incubation at room temperature for 1.5 h, negative stains revealed that many (most) of the assembled actin filaments appeared in bundles (Fig. 4 *b*). Therefore, adding KCl to the extract induced the filaments to form bundles; some transverse order was apparent in these bundles, indicating that the actin filaments, at least in some of the bundles, have their crossover points in register (6).

Assembly of Actin from the Thyone Sperm Extract on Actin Nuclei Isolated from Limulus Sperm

False discharges and KCl to 60 mM were added to the sperm extract immediately after its isolation. 18 min later we took drops of this suspension and negatively stained them (Fig. 5 *a*). We expected to find actin assembled onto the false discharge segments, and since KCl was present, the filaments

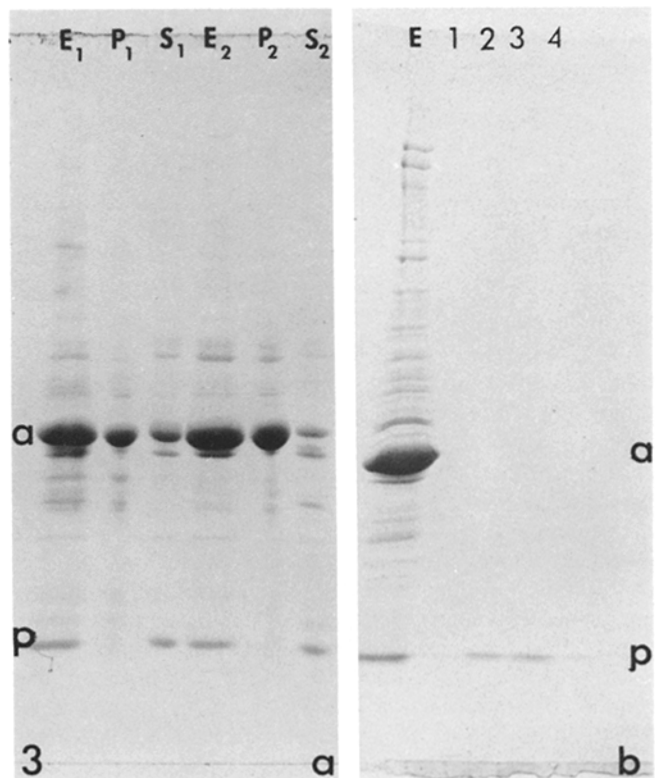


FIGURE 3 (a) SDS PAGE slab gel. The sperm extract, *E* (labeled S_4 in Fig. 2), is made, 60 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris at pH 8.0, then incubated at room temperature for 1 h. The extract is then spun at 100,000 g for 2 h. The resulting pellet, P_1, contains most of the actin (a), but none of the putative profilin (*P*). The supernate (S_1) contains all the putative profilin and <25% of the actin. The experiment was repeated with a different batch of sperm. The sperm extract, E_2, was incubated in the above buffer containing 0.1 mM ATP and spun as before. The pellet (P_2) contained most of the actin, but none of the profilin that is present in the supernate (S_2). (*b*) SDS PAGE slab gel. The sperm extract, *E*, contains two major proteins, actin (*a*) and profilin (*P*). This extract was run over a DEAE column. All the components stick to the DEAE column under the conditions we used except profilin, which comes out in the "void" volume (fractions 1–4). Fractions 2–4 are pooled and used for further assays.

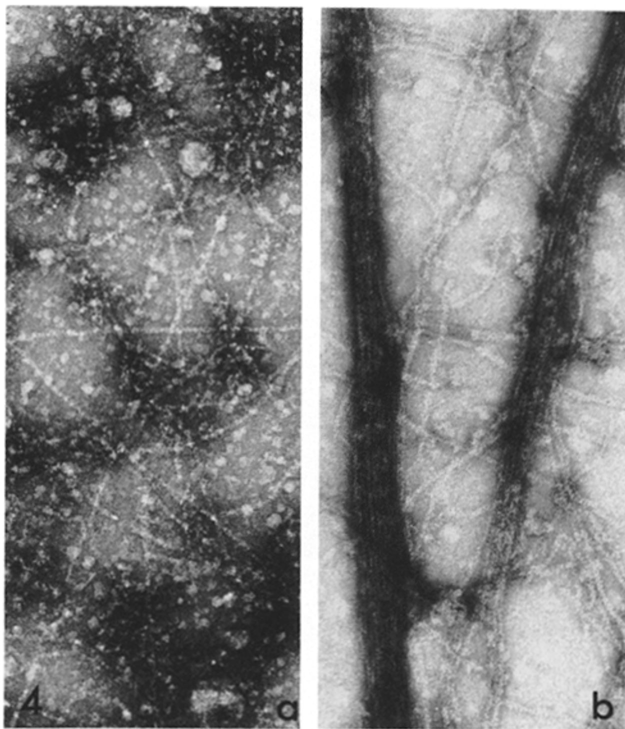


FIGURE 4 (*a*) The sperm extract was incubated in 2 mM $MgCl_2$ and 30 mM Tris at pH 8.0 for 1 h at room temperature, then negatively stained. Randomly arranged filaments are seen. × 140,000. (*b*) The sperm extract containing 60 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris at pH 8.0 was incubated for 1 h at room temperature. The actin polymerizes into filaments, but of particular interest is that most of the actin filaments aggregate to form bundles of filaments. × 140,000.

that assembled from these segments should form bundles. We hoped to see an acrosomal process assembled in vitro. This was, in fact, what we found! What was unexpected and more exciting was that assembly occurred from only one end of the false discharge segment. We found no assembly at all from the other end (Fig. 5 *a*). The concentration of actin in the sperm extract was estimated by determining the total protein concentration in the extract (0.25–0.5 mg/ml) and multiplying this value by the percentage of actin in the extract relative to the other components as determined from the SDS PAGE gels (70%). We obtained a value of 0.17–0.35 mg/ml or an actin concentration of 4–6 μM. To eliminate the possibility that bundling may be an artifact of the assembly conditions, we added 4–6 μM gel-filtered actin to *Limulus* bundles and incubated the bundles under the same conditions as those for the sperm extract, e.g., 2 mM $MgCl_2$, 60 mM KCl, and 30 mM Tris at pH 8.0 for 18 min (Fig. 5 *b*). Unlike the situation in the sperm extract, the newly assembled "pure" actin filaments were not packed into a bundle, but splayed out into a fanlike shape. This control tells us that the actin bundles in the extract must result from the presence of an actin filament cross-linker rather than a preparation artifact. In addition, these actin concentrations and incubation times resulted in filaments nucleating from both ends of the acrosomal bundles. This was in direct contrast with the *Limulus* bundles in the sperm extract, which nucleated filaments from only one end.

As mentioned earlier, the *Limulus* bundle is not only practical for studying actin assembly because of its extreme stability, but because of its taper. By carefully examining the bundle we could determine whether the actin in the extract was preferentially assembling on the preferred end of the bundle, the thin end, or on the nonpreferred end. Invariably the actin assembled on the preferred end (Figs. 6 and 10). In the sperm extract we also found bundles of actin filaments not attached to the *Limulus* bundles that presumably arose from the lateral aggregation of spontaneously assembled actin filaments as well as a small population of free actin filaments.

The length of the process assembled in vitro on the false discharge segments varied from segment to segment, but we have found newly assembled processes that exceeded 9 μm in length. There may even be longer bundles formed since the ends of many cross over onto the grid bars and pipetting, needed for negative staining, will undoubtedly shear the bundles into smaller pieces.

One reviewer of this manuscript was suspicious of our results on the lack of assembly onto the nonpreferred end of the *Limulus* bundle because he felt that we could not detect a very small amount of growth off one end of the *Limulus* bundle. Since other readers may have similar difficulties, we thought we should clarify this point. The *Limulus* bundle consists of a hexagonally packed bundle of actin filaments (7, 21, 26). When the false discharge is isolated, it is sheared by pipetting into pieces 1 μm or so long. The break is sharp. Within the bundle the actin filaments are complexed to two other proteins (21) that not only add mass to the surface of the filaments (e.g., increasing their diameter), but also bind the filaments together to form a paracrystal (7). The actin that assembled from the filaments in the bundle can be readily distinguished from those in the bundle because they are thinner and are not ordered into a paracrystal (Figs. 4 *b*, 7, and 8). Furthermore, actin monomers generally add on the ends of all the filaments in the bundle; it is not possible to miss growth because the filaments preferentially assemble from one side of the bundle, e.g., the top, and "droop down over the end." We estimate that our limit of detection of assembly is <200 Å or 8–10 monomers.

Growth of Sperm Actin on Only the Barbed End of the Limulus Bundles is Not Due to the Presence of a Capping Substance in the Sperm Extract

Initially we entertained the possibility that growth occurs off only one end of the false discharge segment because there is a capping macromolecule present that blocks addition to the nonpreferred or pointed end. We demonstrated with two experiments that this is unlikely. First, we allowed assembly to occur on the *Limulus* bundle for 1 h, then pelleted (10,000 *g* for 10 min) the bundles with their newly assembled filaments, discarded the supernate, resuspended the pellet in buffer, and added 0.1 mg/ml or 2 μM G actin from skeletal muscle. After incubation for 15 min at room temperature, we negatively stained the preparation. We found that actin filaments now extended off both ends of the false discharges. If there were a capping substance present at the nonpreferred end, one would expect no growth on this end when exogenous actin was added. Our second experiment was even more direct. *Limulus* bundles were added to the sperm extract in the presence of 60 mM KCl along with a small volume of monomeric actin. The final concentration of the added muscle actin was 2 μM. The total volume of solution added to

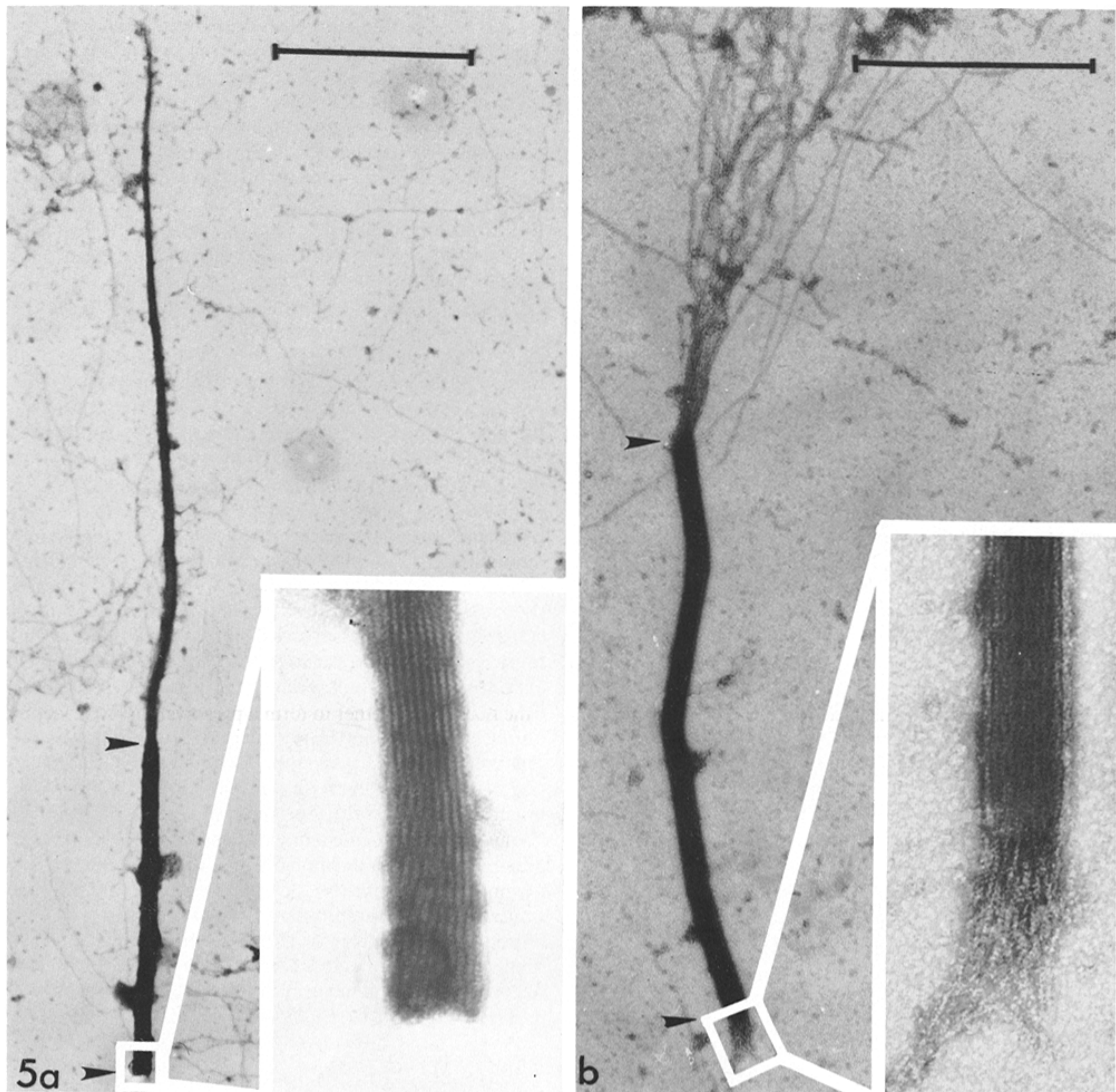


FIGURE 5 (a) *Limulus* acrosomal bundles were added to a sperm extract containing 60 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris at pH 8.0. The solution incubated for 18 min at room temperature at which time it was negatively stained. The arrows indicate the ends of the *Limulus* bundle. Note that assembly has occurred off only one end of the *Limulus* bundle. An inset of the basal end of the bundle, indicated by the small square, shows that no growth at all takes place from the basal end. Also interesting in this figure is that the filaments that extend from the anterior end of the *Limulus* bundle associate laterally to form a bundle. Bar, 1 μm. (a) × 30,000. (*Inset*) × 200,000. (*b*) *Limulus* acrosomal bundles were added to 5 μM gel-filtered actin in 60 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris at pH 8.0. The solution incubated for 18 min at room temperature. Unlike the situation illustrated in *a* with the sperm extract, purified actin assembly takes place at both ends of the bundle (the arrows indicate the ends of the bundle). The basal end is shown at higher magnification in the inset. Also note that the newly assembled filaments splay apart. They do not form a compact bundle. Bar, 1 μm. × 37,500. (*inset*) × 165,000.

the sperm extract did not effectively change the total volume of the extract. Thus, if a capper were present in the supernate or attached to the *Limulus* bundle, it would still be present at essentially the same concentration as in the earlier experiments. We found in both of these experiments that actin assembly occurred off both ends of the bundle (Fig. 6). *Limulus* bundles were incubated for 15 min before negative staining, about the same length of time that the sperm extracts with the *Limulus* bundles were incubated in the preceding section. The above results and those of the previous section, using gel-filtered actin and *Limulus* seeds, discount the possibility that the absence of growth off the nonpreferred end results from a treadmilling mechanism. For such a scheme to occur, the 4–6-μM *Thyone* actin would have to initially assemble onto the nonpreferred end and, as the monomer concentration dropped to below the critical concentration for the nonpreferred end those filaments would then begin to depolymerize. Using 4–6 μM gel-filtered actin we demonstrated that after 18 min the filaments have not depolymerized from the nonpreferred end.

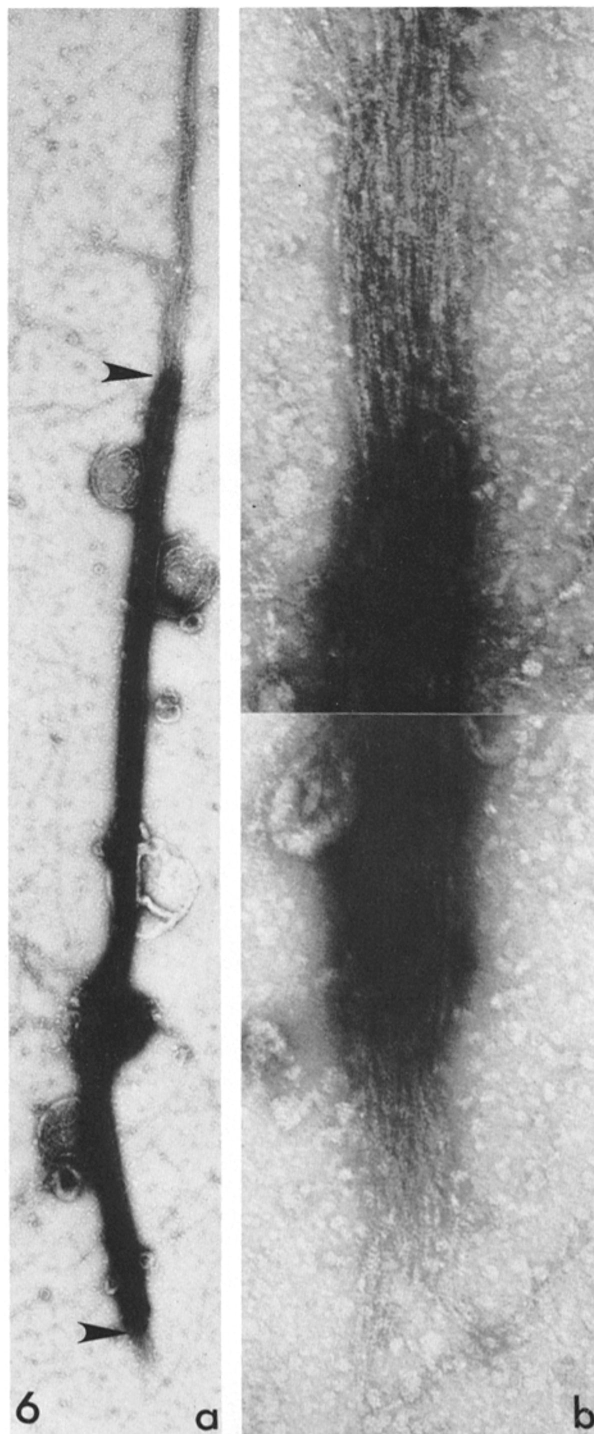


FIGURE 6 *Limulus* acrosomal bundles were added to a sperm extract containing 60 mM KCl, 2 mM $MgCl_2$, and 30 mM Tris at pH 8.0. Immediately, gel-filtered G actin was added to a final concentration of 2 μM. Under these conditions growth occurs off both ends of the bundle, although much more off the apical end than the basal end. The arrows indicate the extent of the *Limulus* bundle. The two ends of the bundle are shown at higher magnification in *b*. (*a*) × 30,000. (*b*) × 170,000.

Isolation and Characterization of the Profilin-like Protein in the Sperm Extract

In an attempt to explain why the actin in the sperm extract assembled on only the barbed end of the *Limulus* bundles, we focused our attention on the other major protein in the sperm extract, the protein labeled *P* in Figs. 2 and 3 *a* (designated P-protein in the following discussion). In particular we suspected that P-protein might be homologous to profilin, a protein first described in extracts of spleen (4), and now found in platelets (8) and *Acanthamoeba* (18, 29). In vitro, profilin inhibits spontaneous nucleation (18), a function that must be carried out during the acrosomal reaction (24). In this section we will demonstrate that P-protein is, in fact, profilin and in a subsequent section show that it inhibits growth on the nonpreferred end.

In Table I we present some of the characteristics of P-protein. We purified it from the sperm extract by DEAE chromatography, making use of the fact that it did not stick to the column, whereas the other proteins did (Fig. 3 *b*). We estimated the molecular weight of the DEAE-purified P-protein by running it on Laemmli gels with appropriate standards in adjacent lanes. The putative sperm profilin ran between hemoglobin and ribonuclease I, indicating that it has a molecular weight of <13,300, yet slightly >12,000. When we plotted log molecular weight against R_f, we estimated that its molecular weight was 12,500. Then, using NEPHGE, the DEAE-purified P-protein was seen to be an extremely basic protein with an apparent isoelectric point of 8.4. Densitometer scans of Coomassie Blue-stained SDS PAGE gels gave a molar ratio of actin to the putative sperm profilin in the sperm extract of ~1:1 (actual values ranged from 1:0.67 to 1:1.3). Finally, preliminary experiments on the effect of the P-protein on the assembly of actin were carried out. When we mixed this protein with G actin and added salt, we found that the rate of assembly of the actin, as measured by viscometry, was much slower than if the P-protein was absent (1 h at 22°C vs. 10 min for muscle actin alone). After 1 h, however, the extent of polymerization was similar to that for the sample containing no profilin. Thus the P-protein in the sperm extract was similar to profilin from both *Acanthamoeba* and spleen with respect to its molecular weight, isoelectric point, molar ratio to actin, failure to bind to DEAE, and effect on actin assembly. Accordingly, we will henceforth refer to this protein as sperm profilin.

Effect of Sperm Profilin on Actin Assembly on the Limulus Nuclei

For these experiments in which we added purified sperm profilin to G actin and examined its effect on the assembly of filaments from the preferred and nonpreferred ends of the *Limulus* bundles, we used actin concentrations, incubation times, and salt and pH conditions in which the rate of filament elongation was linear. We determined the conditions using the analysis of Bergen and Borisy (1) as outlined by Pollard and Mooseker (17). In other words, we incubated the bundles in 0.5 μM actin for 6 min (with and without profilin) in 75 mM KCl and 2 mM $MgCl_2$ buffered to pH 7.5 with 10 mM imidazole. It was most important that the time period was

TABLE I
Characteristics of Sperm Profilin

Mol wt	Isoelectric point	Actin/Profilin
12,500	8.4	1:1

Sperm profilin (*a*) does not bind to DEAE or F actin (*b*) retards actin assembly in vitro.

not arbitrary, but was determined according to the growth rates previously measured (2, 3). Under these conditions most of the actin remained as monomer over the time course of the experiment. We found that when actin (0.5 μM) and profilin (~10 μM) were added to the *Limulus* bundles and then salt added, the actin assembled from only one end of the *Limulus* bundles, the preferred end (Fig. 7 *b*). However, when actin at the same concentration (0.5 μM) was added to the false discharges in the absence of profilin, assembly occurred off both ends (Fig. 7 *a*).

Measurements of the Critical Concentration for the Assembly of Actin on Each End of a Filament Using the False Discharge

Since profilin inhibits the assembly of actin from the pointed end of the false discharge, we examined the minimal concentration of actin necessary for assembly on this end, i.e., the critical concentration at the pointed end. Using the same conditions as outlined in the preceding section, we found that actin (in the absence of profilin) no longer assembled on the pointed end of the false discharge at 0.25 μM (Fig. 7 *c*). By definition the value for the critical concentration at the pointed end of a filament must lie between 0.5 and 0.25 μM. It was actually ~0.37 μM. The critical concentration for the barbed end could then be determined by reducing the concentration of actin added to the false discharge until no assembly occurred at all. This value was 0.1 μM.

It is interesting that this value for the critical concentration changed depending upon the pH, ionic strength, and quality and method of purifying actin. For example, in another batch of freshly gel-filtered actin polymerized under different pH and salt conditions (pH 8.0, 100 mM KCl, and 2 mM $MgCl_2$), the critical concentration for the pointed end was 0.75–1.0 μM, whereas that for the barbed end was 0.1–0.25 μM (Fig. 8). These last experiments were, unfortunately, carried out on samples incubated for 15 min before negative staining. However, similar experiments have been carried out where the incubation time was varied in conjunction with the concentration of monomer in which, as already mentioned, the actin filaments elongated linearly with respect to time. The same critical concentrations were found (2).

DISCUSSION

Two unexpected and fascinating findings were uncovered when we induced filament assembly in the pH 8.0 extract of *Thyone* sperm. First, the newly assembled filaments aggregated laterally to form bundles when KCl was added, indicating that there must be a component present in the extract that cross-linked adjacent filaments. Second, the actin monomers in the sperm extract assembled on only one end of the *Limulus* bundles, whereas purified actin assembled on both ends of the bundles. We determined that the end of the bundle on which assembly occurred was the preferred end for assembly of purified actin in vitro. The absence of assembly to the nonpreferred end was not due to a capping component, but rather to another component, profilin, present in equal molar ratios to actin in the sperm extract.

Bundling of Actin Filaments

From the results of Tilney and Inoué (24) we know that an acrosomal process 70 μm long can form in <7 s. If we examine these processes in thin sections (24) or by negative staining after detergent extraction (unpublished observations), we find a compact bundle of actin filaments, indicating that there must be some component in the extract that cross-links neighboring filaments.

When we induced the assembly of actin from the extract in vitro we also found bundles of actin filaments; however, in the absence of KCl the actin assembled into filaments, but did not form cross-linked bundles. Thus, the cross-linked bundle of actin was not a magnesium paracrystal but rather the result of a component in the extract.

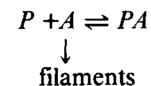
Preliminary observations on the fine structure of the *Thyone* bundles indicate that the filaments in the bundles are not highly ordered, e.g., hexagonally packed, as, for example, are those in the *Limulus* bundles (7). We can determine this lack of order because in negatively stained *Thyone* bundles there were no 1,0 and 1,1 lattice planes. This observation was consistent with the information that on SDS PAGE gels the KCl-induced bundles (Fig. 3 *a*) did not have any major bands other than actin, and suggests that the cross-linker, whatever it may be, was present in a relatively low stoichiometry to actin.

We hope eventually to isolate and characterize this cross-linking substance chemically because it must be capable of cross-linking extremely rapidly. After all, within 7 s the sperm must cross-bridge this elongating bundle of filaments making it rigid enough to pierce the egg jelly.

Regulation of Actin Assembly So That Growth Can Occur Off Only the Preferred End of an Actin Filament

We presented evidence that failure of growth off the nonpreferred end was not due to the presence of a capping substance in the extract. The most direct evidence came from an experiment in which, without effectively diluting the extract, we added 2 μM muscle actin to the sperm extract that contained ~4–6 μM actin. Assembly took place on both the preferred and nonpreferred ends of the bundle. It was difficult to see why increasing the actin concentration by ~30% would somehow induce these hypothetical capping substances to suddenly come off, allowing assembly to take place on the nonpreferred end. Furthermore, if such a capping protein was present in the cell, it would be attached to the basal end of the actomere, an organelle that is not extracted, but remains with the discarded pellet (23).

A more reasonable explanation centers around profilin, a molecule present in a 1:1 molar ratio with actin in the extract that affects the rate of assembly in vitro. One possibility is that profilin binds monomeric actin, thereby lowering the concentration of actin that can assemble. This is depicted as follows:



The amount of free actin, A, in the extract will depend upon the binding constant of profilin to actin and the total concentration of actin and profilin. In this model it is the free actin (A) that assembles onto existing filaments or nuclei, provided that the concentration of A is higher than the critical concentration for assembly. As the actin monomers, A, gradually assembles into filaments, the PA complex disassociates to

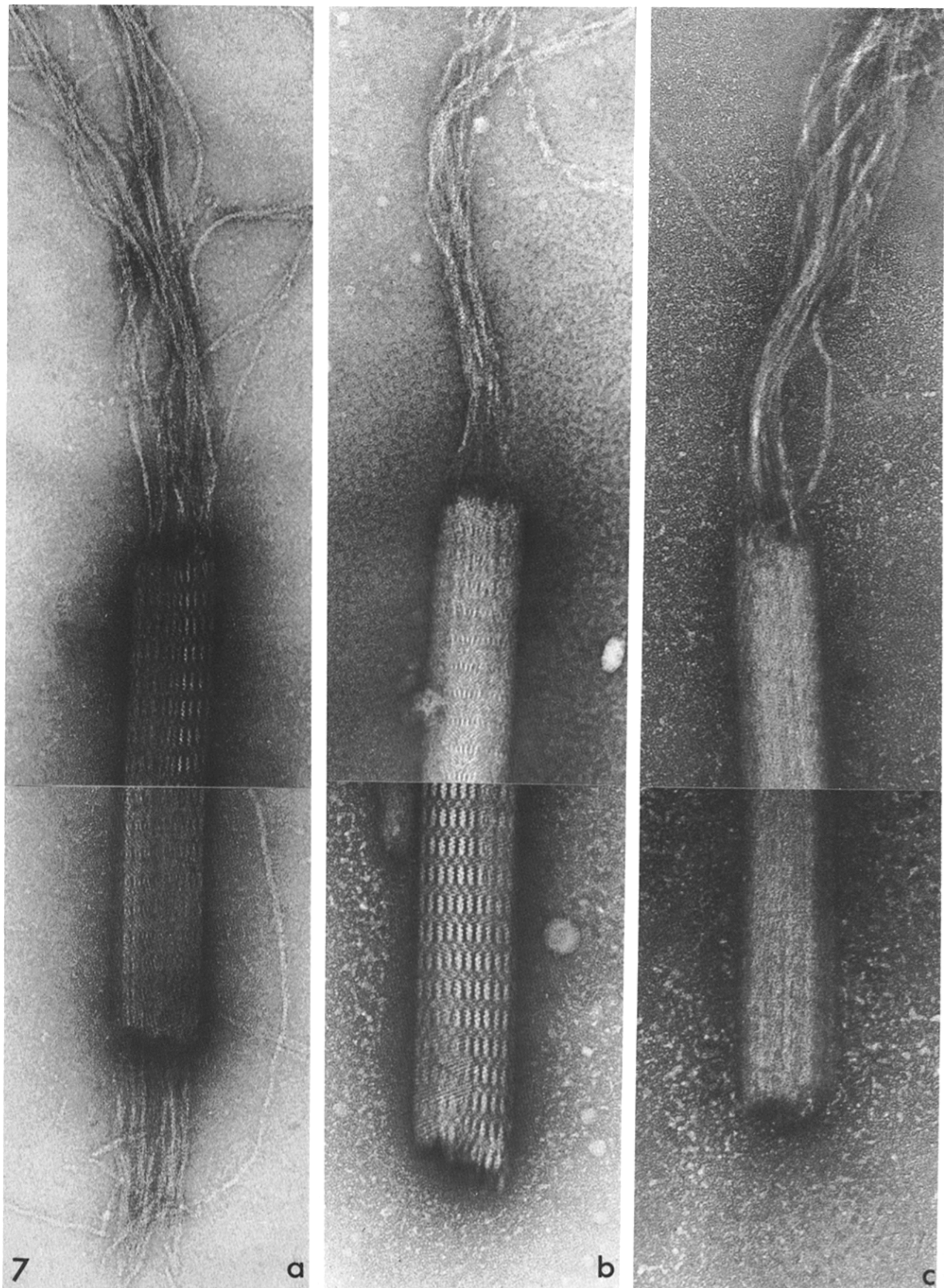


FIGURE 7 (a) Gel filtered G actin was added to the *Limulus* bundles in 75 mM KCl, 3 mM $MgCl_2$, and 10 mM imidazole at pH 7.5, incubated for 6 min, and negatively stained. The final actin concentration was 0.5 μM. The figure is a composite micrograph of the two ends from a single *Limulus* bundle. Note that filament assembly occurred off both ends of the bundle, with more growth off the thinner end. (*b*) The same experiment as in *a*, except in the presence of 10 μM profilin. Note that assembly occurs only off the thinner end of the bundle that corresponds to the preferred end of an actin filament. (c) Nucleated assembly in the presence of 0.25 μM actin monomer. Note that at this actin concentration assembly occurs only from the thinner end of the bundle. × 150,000.

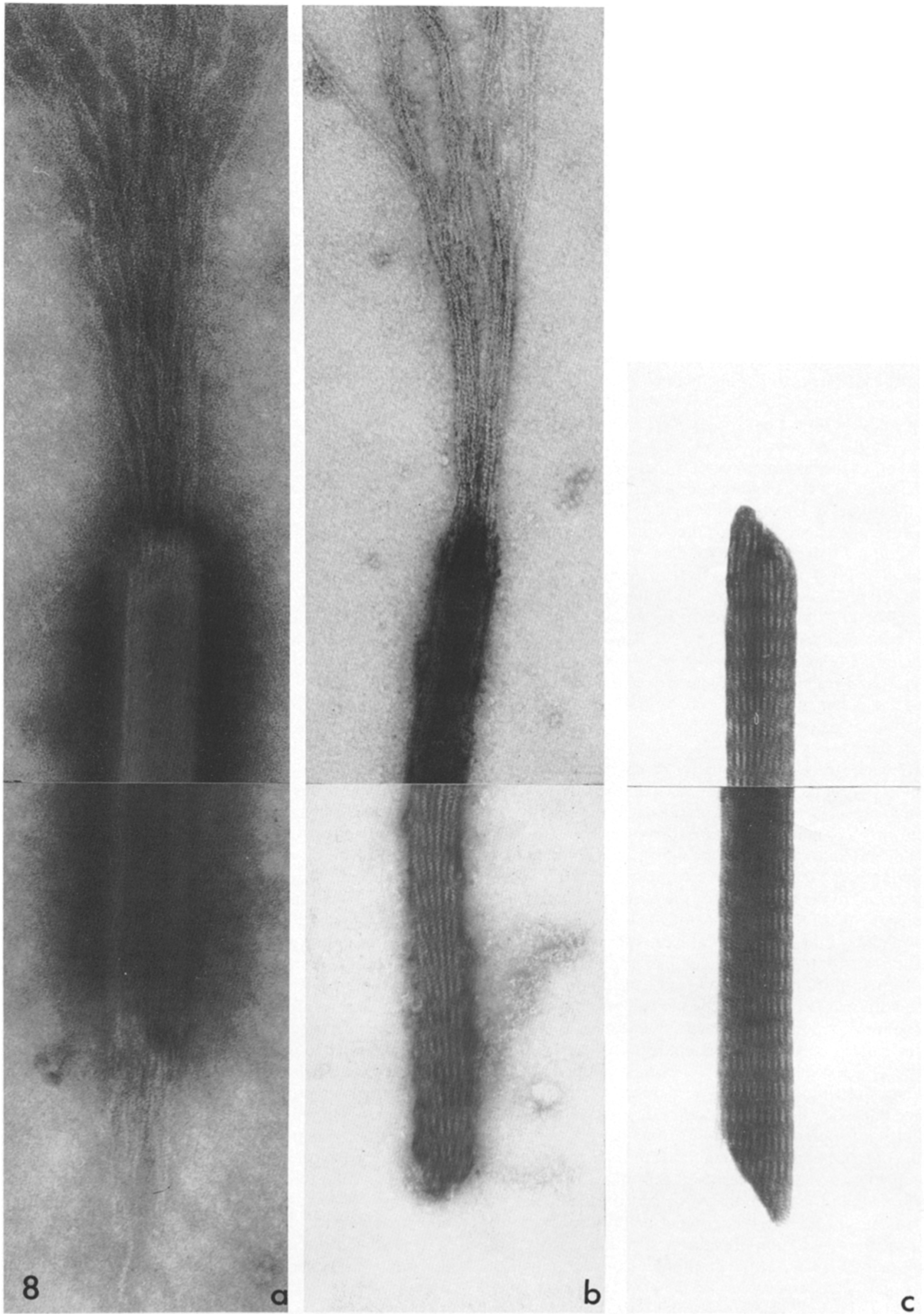


FIGURE 8 *Limulus* acrosomal bundles were added to a solution of G actin containing 30 mM Tris at pH 8.0, and then KCl and $MgCl_2$ were added to give a final concentration of 60 mM and 2 mM, respectively. The two ends of a single bundle are depicted in *a–c*. The thinner or preferred end of the bundle is positioned apically. (*a*) The G actin concentration is 1.5 μM; (*b*) the G actin concentration is 0.5 μM; and (*c*) the G actin concentration is 0.1 μM. × 210,000.

compensate for the loss of *A*, thereby allowing assembly to continue until a new equilibrium is reached. Since the two ends of an actin filament have different critical concentrations, profilin must only maintain the concentration of free actin, *A*, between the two values for the critical concentration to achieve unidirectional addition of monomers. For example, Fig. 8 demonstrates that the critical concentration for the preferred end is 0.1 μM, whereas at the nonpreferred end it is 1 μM. Thus, under these conditions the critical concentration for the two ends differs by ~10-fold. Therefore, when profilin maintains the free actin concentration, *A*, at >0.1 μM but <1 μM, actin monomers add only to the preferred end with no assembly from the other end. In this model profilin is, in essence, a buffer that regulates the amount of polymerizable actin. The beauty of this mechanism is that profilin limits filament elongation to the preferred end by using the intrinsic difference in critical concentrations at the ends of an actin filament, e.g., the polarity of the filament.

Although this model fits the in vitro data, it cannot be used to explain what occurs in vivo, as was kindly pointed out to us by Tom Pollard. Briefly stated, we know from the rate of elongation of the acrosomal process that the filaments must be elongating at a rate of 400 molecules/s (24). Extrapolating from the studies of Pollard and Mooseker (17), in which they plotted the rate of assembly as a function of actin concentration, we found that to support the filament assembly rate we measured in vivo requires an actin concentration at the growing tip of 40 μM, (17). An even higher concentration, in excess of 4,000 μM, must be present at the base of the process to drive the monomers to the tip (24). This is precisely the concentration of actin estimated to be in the sperm prior to the acrosomal reaction (24). Thus, if profilin acts by maintaining the polymerizable actin concentration at 0.1–1 μM, the acrosomal process could not form at the rates that have been measured (24).

Fortunately, there is an alternative model that accounts for the elongation of the acrosomal process in the sperm. Moreover, this model can also be used to explain the behavior of profilin in vitro. Our idea is diagrammed in Fig. 9. In this model the profilin, *P*, binds to one side of an actin monomer, *a*, forming a *PA* complex. This complex can bind to the preferred end of the bundle, since the *b* site of the monomer can bind to the *a* site of the filament. Upon binding, the conformation of the newly bound monomer is changed and *P* falls off. In contrast, the PA complex cannot bind to the nonpreferred end of the filament because the profilin is blocking the *a* site on the monomer, the site that would bind to the *b*, or nonpreferred end of the actin filament. The rate that actin assembled on the preferred end is limited only by the off rate of the profilin (*P*). The *P* liberated during assembly grabs any free *A* and forms a *PA* complex. Furthermore, if any uncomplexed, monomeric actin exists, it would tend to assemble on the preferred end of existing filaments since the critical concentration on this end is 10 times higher than on the nonpreferred end. The beauty of this model is that when the off rate of profilin from the newly added *PA* complex on the filament is rapid, then the rate of assembly is proportional to the total concentration of actin present, not just the free actin. Briefly, then, 40 μM actin-profilin should assemble at approximately the same rate as would 40 μM free actin. Once the actin starts to polymerize, profilin is released, which in turn ties up any free actin. The consequences of this insures that spontaneous nucleation is inhibited and assembly occurs on only the preferred end of filaments located at the tip of the process.

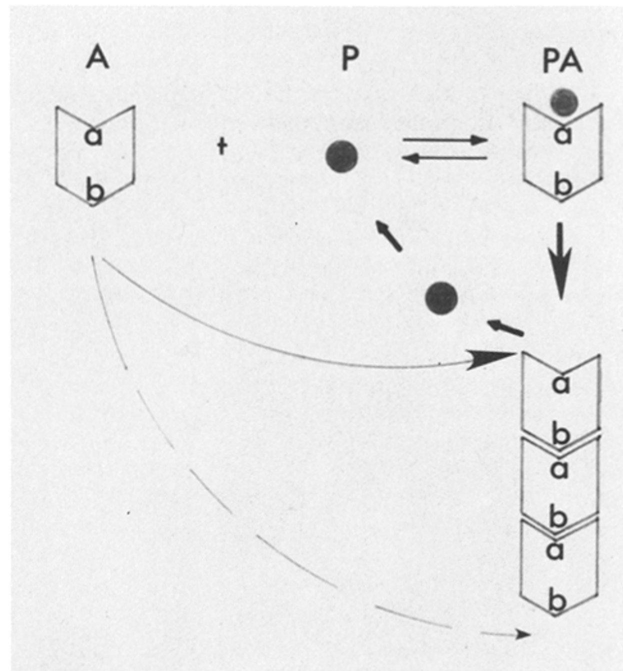


FIGURE 9 Proposed mechanism of profilin behavior. Profilin, *P*, (the black circle) binds to the *a* site of an actin monomer, *A*, forming a *PA* complex. Although actin by itself can bind either end of an actin filament, binding of profilin causes the conformation of the actin monomer to change such that addition to the growing filament is at only one end, the preferred end (the "*a*" end). Upon addition of the monomer to the filament, the profilin molecule is released and can now intereact with another actin monomer. The "*b*" site is the nonpreferred end for monomer addition. This model provides for unidirectional growth of the actin filament.

To our knowledge this model has not been suggested before. Nevertheless, when we carefully examined the published literature, we found that not only is our model consistent with all the available data, but also there are at least three places where our model explains some previously puzzling observations. First, Tobacman and Korn (28) demonstrated that once *Acanthamoeba* profilin-actin starts to polymerize, the rate of assembly is surprisingly similar to the rate in control preparations without profilin (Fig. 1 in reference 28). Although profilin inhibits the rate of nucleation, thereby delaying the onset of filament elongation, it does not significantly inhibit polymerization once it starts. Assuming that the initial rates are proportional to filament elongation (13, 17), the profilin-actin complex polymerizes at a rate only slightly less than the controls. Furthermore, since the rate of filament elongation is dependent upon the monomer concentration, their experiments suggest that most of the actin is available to polymerize, not only the free monomer. The slight decrease in assembly rate may occur because the actin pool cannot assemble from the nonpreferred end of the filaments. Second, using *Acanthamoeba* profilin, Tseng and Pollard (29) have most convincingly demonstrated that profilin does not affect elongation from the preferred end of an actin filament (Fig. 4 of reference 29). In 50 mM KCl and 1 mM $MgCl_2$, they show that profilin-actin polymerizes off the preferred end of an S1 decorated actin filament at the same rate as in controls. Under the same conditions the initial rate of seeded actin polymeri-

zation is inhibited in profilin-containing solutions as judged by viscometry (Fig. 3 *c* of reference 29). This indicates that the profilin must be inhibiting growth from the nonpreferred end of the filaments, whereas the preferred end grows at a rate dependent on the total monomer concentration. Third, Markey et al. (14), in a series of experiments, examined filament elongation in the presence of profilin using nucleating seeds that can grow filaments only from the preferred end. When spectrin-actin-4.1 complex is used as the nucleating seed, both the profilin-actin and actin controls assemble at similar rates (Fig. 2 of reference 14). This nucleating site presumably only exhibits preferred end filament growth. Again, this indicates that the total concentration of actin is available to polymerize, not only free A from the profilin-actin equilibrium. When villin, which caps the preferred end of an actin filament, thereby limiting filament elongation to the nonpreferred end (3), is added to the profilin-actin solutions, actin polymerization is even further inhibited. The nonpreferred ends do not start assembling actin filaments, indicating that the concentration of monomer competent to polymerize from this end must be low (Fig. 5 of reference 14). These two pieces of data suggest that the preferred end of the filament elongates at a rate equal to the total monomer, whereas the nonpreferred end sees a concentration that is much lower. This indicates that the profilin-actin assembles from the preferred end as if the total actin concentration is available, whereas the nonpreferred end grows as if it only sees the free monomer.

Our experiments make it possible to obtain a rough estimate for the K_d of the sperm profilin-actin complex. In our model of profilin function it was only the free (unbound) monomer that could add onto the nonpreferred filament end. The concentration of this pool was, then, easily determined since previously it has been shown that the rate of assembly from the ends of the bundle is linearly dependent upon the initial concentration of G actin (2). When 0.5 μM G actin was added to the bundles in the presence of $\sim$10 μM profilin, the actin assembled off the bundles as if there was an initial free monomer concentration of $<$0.37 μM, not 0.5 μM. Therefore, the presence of profilin decreased the free actin concentration by $\sim$0.13 μM (the difference between 0.5 and 0.37 μM). Assuming that this decrease resulted from profilin binding actin at a 1:1 ratio, the free profilin concentration was 9.87 μM. Using these values we got a rough estimate for the K_d of $\sim$10 μM. This estimate closely agrees with the value calculated for *Acanthamoeba* profilin (28, 29). This K_d was estimated for sperm profilin and muscle actin.

Importance of These Findings in Elucidating the Mechanism for Actin Assembly in the Formation and Elongation of the Acrosomal Process In Vivo

Our observation that the assembly of actin monomers from the sperm extract occurred on only the preferred end of the filaments in vitro is consistent with earlier studies on the effects of cytochalasin and the kinetics of elongation of the acrosomal process (24). From those studies we concluded that actin monomers add to the ends of the filaments located at the tip of the acrosomal process. At the same time, if monomers of actin are to diffuse to the tip of the acrosomal process before assembly, there must be a component present that limits the amount of spontaneous nucleation (24), yet allows diffusion of monomers to the tip of the acrosomal process at essentially the same rate. Profilin, because of its small size, is a good candidate for this. At the same time, profilin must not alter the rate of assembly on the preferred end of existing filaments. Our results on the assembly of actin with sperm profilin indicated that this protein could indeed play such a role, simultaneously inhibiting nucleation and allowing elongation to occur at normal rates.

Our in vitro study also focused our attention on two other facts in the formation and elongation of the acrosomal process in vivo. First, it is clear that there must be an actin cross-linker that binds adjacent actin filaments together as they elongate. It must be an interesting substance since it must work rapidly (e.g., within 7 s). Second, although profilin appears to act to lower the level of spontaneous nucleation and influences to which end the monomers add, it cannot be the component that inhibits assembly in the untreated sperm. What is needed there is not only a complete inhibition of actin assembly but also a substance that binds and thus sequesters the actin in situ in the profilactin region in a nondiffusible form. It is likely that the high molecular weight substances previously identified by Tilney (22) play a role here.

We would especially like to express our gratitude to Annemarie Weber who, over a memorable breakfast, told one of us (L. G. Tilney) what our results might mean and in subsequent discussions tried to get us to think coherently. She also kindly supplied the gel-filtered actin for one of the experiments reported here. We would particularly like to express our thanks to Tom Pollard, a reviewer of this manuscript, who pointed out to us that our preliminary model was inconsistent with the kinetics of elongation of the acrosomal process, and to James Spudich, another reviewer, who, along with Annemarie Weber, got one of us (L. G. Tilney) finally thinking about rate constants. We also wish to thank Joel Rosenbaum for the use of his electron microscope.

This work was supported by National Institutes of Health (NIH) grant HD 14474 (L. G. Tilney and L. Coluccio), NIH training grant T32 GM07229-06 E. M. Bonder, and NIH grant AM 25387 (M. S. Mooseker).

Received for publication 27 September 1982, and in revised form 14 March 1983.

REFERENCES

1. Bergen, L. G., and G. G. Borisy. 1980. Head-to-tail polymerization of microtubles in vitro. Electron microscope analysis of seeded assembly. *J. Cell Biol.* 84:141–150.
2. Bonder, E. M., and M. S. Mooseker. 1981. The acrosomal process: a new tool in studying actin assembly and structure. *J. Cell Biol.* 91(2, Pt. 2):306*a*. (Abstr.)
3. Bonder, E. M., and M. S. Mooseker. 1983. Direct electron microscopic visualization of barbed end capping and filament cutting by intestinal microvillar 95-kdalton protein (villin): a new actin assembly assay using the *Limulus* acrosomal process. *J. Cell Biol.* 96:1097–1107.
4. Carlson, L., L. E. Nystrom, I. Sundvist, F. Markey, and U. Lindberg. 1977. Actin polymerization is influenced by profilin, a low molecular weight protein in non-muscle cells. *J. Mol. Biol.* 115:465–483.
5. Craig, S. W., and T. D. Pollard. 1982. Actin-binding proteins. *Trends Biochem. Sci.* 7:88–92.
6. DeRosier, D. J., and L. G. Tilney. 1982. How actin filaments pack into bundles. *Cold Spring Harbor Symp. Quant. Biol.* 46:525–540.
7. DeRosier, D. J., E. Mandelkow, A. Silliman, L. Tilney, and R. Kane. 1977. The structure of actin-containing filaments from two types of non-muscle cells. *J. Mol. Biol.* 113:679–695.
8. Harris, H. E., and A. G. Weeds. 1978. Platelet actin: subcellular distribution and association with profilin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 90:84–88.
9. Inoué, S., and L. G. Tilney. 1982. The acrosomal reaction of *Thyone* sperm. I. Changes in the sperm head visualized by high resolution video microscopy. *J. Cell Biol.* 93:812–819.
10. Korn, E. D. 1982. Actin polymerization and its regulation by proteins from non-muscle cells. *Physiol. Rev.* 62:672–737.
11. Laemmli, V. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.
13. MacLean-Fletcher, S., and T. D. Pollard. 1980. Identification of a factor in conventional

muscle actin preparations which inhibit actin filament self associations. *Biochem. Biophys. Res. Commun.* 96:18–27.
14. Markey, F., H. Larsson, K. Weber, and U. Lindberg. 1982. Nucleation of actin polymerization from profilactin opposite effects of different nuclei. *Biochim. Biophys. Acta.* 704:43–51.
15. Matsudaira, P. T., and D. R. Burgess. 1978. SDS microslab-linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem.* 87:386–396.
16. O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two dimensional electrophoresis of basic as well as acidic proteins. *Cell.* 12:1133–1142.
17. Pollard, T. D., and M. S. Mooseker. 1981. Direct measurement of actin polymerization rate constants by electron microscopy of actin filaments nucleated by isolated microvillus cores. *J. Cell Biol.* 88:654–659.
18. Reichstein, E., and E. D. Korn. 1979. *Acanthamoeba* profilin. A protein of low molecular weight from *Acanthamoeba castellanii* that inhibits actin nucleation. *J. Biol. Chem.* 254:6174–6179.
19. Schackmann, R. W., and B. M. Shapiro. 1981. A partial sequence of ionic changes associated with the acrosome reaction of *Strongylocentrotus purpuratus*. *Dev. Biol.* 81:145–154.
20. Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.
21. Tilney, L. G. 1975. Actin filaments in the acrosomal reaction of *Limulus* sperm. Motion generated by alterations in the packing of the filaments. *J. Cell Biol.* 64:289–310.
22. Tilney, L. G. 1976. The polymerization of actin. III. Aggregates of nonfilamentous actin and its associated proteins: a storage form of actin. *J. Cell Biol.* 69:73–89.
23. Tilney, L. G. 1978. Polymerization of actin. V. A new organelle, the actomere, that initiates the assembly of actin filaments in *Thyone* sperm. *J. Cell Biol.* 77:551–564.
24. Tilney, L. G., and S. Inoué. 1982. Acrosomal reaction of *Thyone* sperm. II. The kinetics and possible mechanism of acrosomal process elongation. *J. Cell Biol.* 93:820–827.
25. Tilney, L. G., and N. Kallenbach. 1979. Polymerization of actin. VI. The polarity of the actin filaments in the acrosomal process and how it might be determined. *J. Cell Biol.* 81:608–623.
26. Tilney, L. G., E. M. Bonder, and D. J. DeRosier. 1981. Actin filaments elongate from their membrane-associated ends. *J. Cell Biol.* 90:485–494.
27. Tilney, L. G., D. P. Kiehart, C. Sardet, and M. Tilney. 1978. Polymerization of actin. IV. The role of Ca^{++} and H^+ in the assembly of actin and in membrane fusion in the acrosomal reaction of echinoderm sperm. *J. Cell Biol.* 77:536–550.
28. Tobacman, L. S., and E. D. Korn. 1982. The regulation of actin polymerization and the inhibition of monomeric actin ATPase activity by *Acanthamoeba* profilin. *J. Biol. Chem.* 257:4166–4170.
29. Tseng, P. C.-H., and T. D. Pollard. 1982. Mechanism of action of *Acanthamoeba* profilin: demonstration of actin species specificity regulation by micromolar concentrations of $MgCl_2$. *J. Cell Biol.* 94:213–218.
30. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406–4412.
31. Woodrum, D. T., S. A. Rich, and T. D. Pollard. 1975. Evidence for biased bidirectional polymerization of actin filaments using heavy meromyosin prepared by an improved method. *J. Cell Biol.* 67:231–237.