ACTIN IN TRITON-TREATED CORTICAL PREPARATIONS OF UNFERTILIZED AND FERTILIZED SEA URCHIN EGGS

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ABSTRACT

Triton-treated cortical fragments of unfertilized and fertilized sea urchin eggs prepared in the presence of ≥5 mM EGTA contain 15–30% of the total egg actin. However, actin filaments are not readily apparent by electron microscopy on the cortical fragments of unfertilized eggs but are numerous on those of fertilized eggs. The majority of the actin associated with cortical fragments of unfertilized eggs is solubilized by dialysis against a low ionic strength buffer at pH 7.5. This soluble actin preparation (<50% pure actin) does not form proper filaments in 0.1 M KCl and 3 mM $MgCl_2$, whereas actin purified from this preparation does, as judged by electron microscopy. Optical diffraction analysis reveals that these purified actin filaments have helical parameters very similar to those of muscle actin. Furthermore, the properties of the purified actin with regard to activation of myosin ATPase are similar to those of actin from other cell types. The possibility that actin is maintained in a nonfilamentous form on the inner surface of the unfertilized egg plasma membrane and is induced to assemble upon fertilization is discussed.

KEY WORDS sea urchin egg · Triton-treated cortical fragments · actin · fertilization · microfilaments

The assembly and organization of the contractile apparatus of nonmuscle cells is under highly regulated controls (for reviews, see references 10, 14, 52). A classical case in point is the demonstration by Schroeder (38, 39) that a "contractile ring" of actin filaments appears in what becomes the furrow region of the cell membrane just before cell division. There are many other situations where bundles of actin filaments transiently assemble to suit a particular need of the living cell, and studies at the molecular level regarding the controls imposed on this assembly of actin filament bundles is presently in its infancy. We use the term "bundle" here very generally, although there are clearly different types of actin filament bundles in cells. However, very little is known about the molecular mechanism of formation of any type of bundle.

Ideally, to study the transformation of actin from some nonfilamentous state to the form of actin filament bundles, a cell type should be studied which has the following characteristics: (*a*) the cells and the number of bundles formed should be abundant to provide enough material for biochemical analysis; (*b*) bundle formation should be able to be experimentally induced; and (*c*) bundle formation should occur synchronously in the cell population. Sea urchin eggs fulfill all these criteria. Both unfertilized and fertilized eggs have a distinct

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Volume 82 July 1979 212–226

layer or cortex just beneath the plasma membrane (for reviews, see references 18, 27, 36). Cortical preparations have been made from lysed fertilized eggs and examined biochemically by Sakai (37) and Mabuchi and Sakai (26). These authors did not report the presence of actin in their cortical preparations, but Burgess (7) and Begg et al. (2) have reported that actin is a major component of cortices isolated from fertilized eggs, as judged by polyacrylamide gel electrophoresis. Filaments are not readily apparent in the cortex of unfertilized eggs (3, 28). Upon fertilization there is synchronous elongation of thousands of microvilli on each egg. This elongation of microvilli is complete before the first cell division (8, 12, 40). Harris (15) observed microfilaments in the cortex of fertilized eggs of *Strongylocentrotus purpuratus*, and Burgess and Schroeder (8) recently showed that the microvilli of fertilized *S. purpuratus* eggs contain actin filaments in the form of a bundle, which has an ~120 Å transverse periodicity presumably due to an accessory component bound to the filament array. The detailed substructure of these microvillar filament bundles has been examined by high-resolution electron microscopy and diffraction analysis by Spudich and Amos (44). The transverse periodicity reflects the positions of cross-bridges that connect the filaments into a bundle. Diffraction analysis reveals that there are ~14 actin monomers between cross-overs of the two long-pitch helical strands of the actin filaments with three cross-bridges in this interval.

Changes in the state of the actin which may occur upon fertilization can be studied biochemically and structurally since eggs can be obtained in large quantities. Actin has been isolated and characterized from a variety of species of sea urchin eggs (17, 21, 29–31). Mabuchi and Spudich[1] have purified and characterized actin found in the supernate after high speed centrifugation of lysed unfertilized eggs of *S. purpuratus*. That pool of actin has properties very similar to those of muscle actin when in the purified state, but does not readily polymerize in the crude extract. The microvillar actin filaments of fertilized eggs could derive from this soluble, cytoplasmic actin pool. However, as we describe here and have reported earlier (47), Triton-treated cortical preparations of unfertilized eggs contain a significant population of actin which may be in a nonfilamentous form. It is possible that this pool of actin is the immediate precursor to the microvillar filament bundles. Indeed, Begg and Rebhun (3) have reported that cortices isolated from unfertilized eggs by lysis in a buffer without Triton X-100 at pH 6.5 do not contain microfilaments, but that a dense network of microfilaments appears upon raising the pH to 7.5. They propose that the increase in cytoplasmic pH from ~6.4 to ~7.3 that occurs upon fertilization (20, 24, 42) results in polymerization of actin associated with the cortex and concomitant formation of microvilli. These concepts are in keeping with those first proposed by Tilney et al. (50) in their studies on the acrosomal reaction of echinoderm sperm.

[1] Mabuchi, I., and J. A. Spudich. Manuscript in preparation.

MATERIALS AND METHODS

General Procedures

Gametes from *S. purpuratus* were used for all experiments described here. Eggs were shed by intracoelomic injection of 0.5 M KCl and collected into Millipore-filtered sea water at 15°C. They were passed through bolting silk and washed three times by settling out of a large excess of Millipore-filtered seawater. The washed eggs were packed by hand centrifugation and resuspended in appropriate solutions to the required volumes (see below).

SDS-PAGE[2] was done according to the discontinuous system of Laemmli (22), as modified by Ames (1). Electron microscopy was carried out on samples negatively stained with 1% uranyl acetate (19). Grids were examined in a Philips 300 electron microscope. Optical diffraction analysis was carried out as described by DeRosier and Klug (11). Protein determinations were done according to the method of Lowry et al. (25), as modified by Hartree (16). All procedures were carried out at ~22°C, unless otherwise noted.

Preparation of Triton-Treated Cortical Fragments from Unfertilized and Fertilized Eggs Attached to Polycation-Coated Surfaces

Washed and packed unfertilized eggs were suspended in 5 vol of isotonic salt solution (0.5 M NaCl, 0.026 M KCl) and allowed to settle for 3 min on acid-washed glass petri dishes coated with either 1 mg/ml polylysine

[2] Abbreviations used in this paper: SDS, sodium dodecyl sulfate; MES, 2[N-morpholino]ethane sulfonic acid; EGTA, ethyleneglycol-bis-(β-amino ethyl ether) N,N'-tetra-acetic acid; TAME, *p*-tosyl-L-arginine methyl ester; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.

(Sigma Chemical Co., St. Louis, Mo.) or 10 mg/ml protamine sulfate (Calbiochem Behring Corp., San Diego, Calif.). Unattached eggs were rinsed away with salt solution, and attached eggs were lysed by squirting a stream of shearing solution (0.1 M MES, 5 mM EGTA, 10 mM TAME, 1 mM $MgCl_2$, pH 6.8) containing 0.5% Triton X-100 across the surface from a wash bottle (6, 8, 9, 53). The shearing solution was adapted from that used by Rebhun et al. (34) for the isolation of mitotic apparatus from *Spisula* eggs. Each dish was then rinsed with the same buffer without Triton X-100. All of the above operations were carried out at 15°C. The attached egg fragments that remained on the petri dish were apparent by light microscopy.

In other experiments, washed eggs were treated with 10 mM DTT at pH 9.0 for 3 min before fertilization to prevent the subsequent formation of fertilization membrane (13). The eggs were then fertilized, washed free of sperm with isotonic salt solution and put down on petri dishes coated with 1 mg/ml polylysine or 10 mg/ml protamine sulfate. These eggs were allowed to develop as a monolayer in Millipore-filtered seawater at 15°C, and were lysed and washed at various times after fertilization as described above for unfertilized eggs.

Preparation of Triton-Treated Cortical Fragments for Electron Microscopy

Triton-treated cortical fragments of unfertilized and fertilized eggs were prepared as described above, except that polylysine-coated electron microscope grids were used instead of petri dishes and the shearing solution with Triton X-100 was directed over the surface of the grid from a syringe. The washed cortical fragments were stained with 1% uranyl acetate.

Quantitation of the Amount of Actin on Triton-Treated Cortical Fragments of Unfertilized and Fertilized Eggs

In general, actin was quantitated from scans of the Coomassie-stained band that comigrated with purified muscle actin on SDS-polyacrylamide gels. This method appears justified since the major peak that comigrates with purified muscle actin is all retained on a DNase I column in the presence of 0.6 M KI (Fig. 1), as expected for actin (5, 23).

The amount of actin on the cortical fragments that remained attached to the petri dishes after shearing was determined as follows. The contents of each dish were dissolved in 30 mM Tris, pH 6.8, 1% SDS, 5% glycerol, and 2.5% β-mercaptoethanol, and an aliquot was electrophoresed on polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue and actin was quantitated by scanning the gels with a Transidyne RFT Scanning Densitometer (Transidyne General Corp., Ann Arbor, Mich.) at 550 nm; the peak corresponding to actin was cut out and weighed. The peak area was converted to milligrams of actin using a standard curve prepared with purified muscle actin. When quantitating actin from samples containing many other proteins, the baseline of the peak was selected to account for the contribution from other staining material (see dotted line in Fig. 1).

The total apparent surface area[3] of egg fragments retained on the petri dish was determined by multiplying the total number of fragments by the average apparent surface area (2,800 ± 780 μm^2, 236 measurements) of a patch measured using an ocular micrometer. The amount of actin per square micron of apparent surface area was then calculated by dividing the total milligrams of actin from the petri dish by the total apparent surface area of the fragments retained on the dish.

Nonspecific sticking of actin to the polycation-coated petri dishes was insignificant. In control experiments, egg lysates were prepared in the Triton-containing shearing solution and then were incubated with polycation-coated petri dishes. The dishes were rinsed with the same buffer without Triton X-100. The contents of each dish were then dissolved as described above and analyzed by PAGE for actin, and none was apparent.

The total amount of actin per unfertilized egg (2×10^{-4} μg) was determined by dissolving a known number of eggs in 30 mM Tris, pH 6.8, 1% SDS, 5% glycerol, and 2.5% β-mercaptoethanol and quantitating the actin content by PAGE, as described above.

Preparation of Triton-Treated Cortical Fragments in Suspension and Purification of Cortical Actin

For biochemical analysis, egg cortical fragments were isolated as follows (Fig. 2). Washed and packed unfertilized eggs were suspended in 10 vol of shearing solution with Triton X-100 and lysed by brief vortexing, or for large volumes by gentle shearing in a Waring blender. The lysed egg suspension was then centrifuged at 1,600 *g* for 10 min. The pellet (P1) was washed once with 5 vol of shearing solution with Triton X-100 (P2) and then with 5 vol of shearing solution without Triton X-100 (P3; 20% of the actin of the egg).

Actin was released from P3 by overnight dialysis against G-buffer (10 mM triethanolamine, 1 mM DTT, 0.2 mM $MgCl_2$, 0.2 mM ATP, pH 7.5) at 4°C. Dialysis was followed by high speed centrifugation at 100,000 *g*

[3] We define "apparent surface area" as the area of an attached egg cortical fragment determined using an ocular micrometer in a phase-contrast light microscope. The diameter of the fragment was measured, and the area was calculated assuming a circular fragment. The "apparent surface area" does not correspond to the true surface area (41) since folds and microvilli are ignored in the former. The total number of egg fragments retained on the petri dish was determined by counting them in a phase-contrast light microscope.

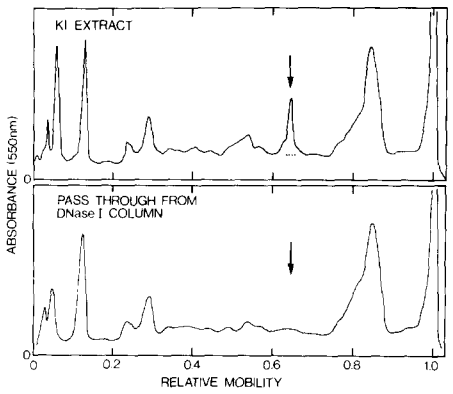

FIGURE 1 Scans of SDS-polyacrylamide gels of whole egg extract and the pass through of the whole egg extract on a DNase I Sepharose column. Unfertilized eggs were washed in Millipore-filtered seawater, and 2 ml of packed eggs were lysed by sonication into 10 ml of a solution containing 0.6 M KI in G-buffer. The homogenate was then centrifuged at 100,000 *g* for 1 h at 4°C. All of the protein of 42,000 mol wt was in the supernate as observed by PAGE. An aliquot (0.2 ml) of the high-speed supernate (KI extract) was loaded onto a DNase I Sepharose column (0.3 ml of a DNase I Sepharose suspension (Worthington Biochemical Corp., Freehold, N. J.) was placed in a 1 ml syringe on top of a small column of glass wool, and the column was rinsed at 4°C with 2 ml of 3 M guanidine-HCl and then with 2 ml of 0.6 M KI in G-buffer). The sample was incubated in the column for 30 min at 4°C. The column was then washed with 1.2 ml of 0.6 M KI in G-buffer (pass through). The arrows show where purified skeletal muscle actin runs on identical gels. The major 42,000 dalton component in the KI extract is quantitatively retained on the DNase I Sepharose column as expected for actin (5).

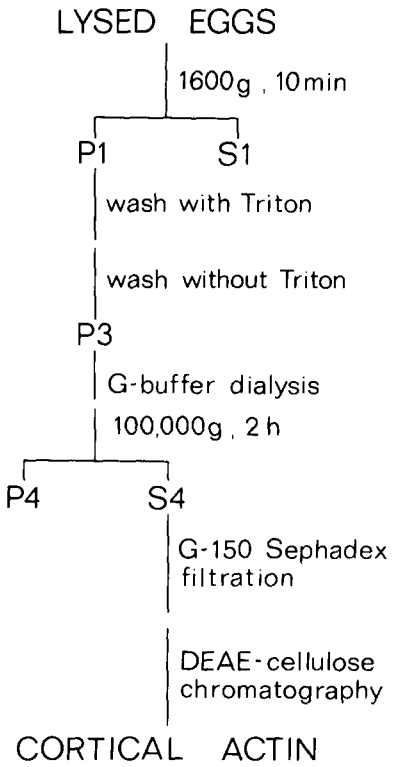

FIGURE 2 A flow diagram of the steps followed for obtaining cortical actin from unfertilized eggs. See Materials and Methods for details.

for 2 h. The supernate (S4; 75% of the actin in P3) was fractionated at 4°C on a Sephadex G-150 column and the actin peak was pooled and chromatographed on a DEAE-cellulose column, using the buffer conditions described by Uyemura et al. (51) for *Dictyostelium* actin, with modifications for the smaller volumes used in the experiments reported here. The actin peak from the DEAE column was pooled (cortical actin; 10% of the actin in S4).

RESULTS

Quantitation of Actin on Triton-Treated Cortical Fragments from Unfertilized Sea Urchin Eggs

About 20% of the total actin of unfertilized eggs was found to be associated with Triton-treated cortical fragments in two types of experiments. In one type of experiment, eggs that had adhered to polycation-coated petri dishes were sheared with a buffer containing Triton X-100. The unfertilized egg cortical fragments left attached to the dish were found to contain $\sim 2 \times 10^{-9}$ μg actin/μm^2 apparent surface area (values ranged from 1.5×10^{-9} to 3.0×10^{-9} in five experiments, with an average value of 2.2×10^{-9}). Using 80 μm as the diameter of an egg, the total apparent surface area is 2×10^4 μm^2. Assuming that the fragment of egg surface that remains attached to the dish is representative of the total egg surface, the cortical preparation contains $\sim 4 \times 10^{-5}$ μg actin/egg or 20% of the total egg actin. Nonspecific sticking of actin to the polycation-coated petri dishes was insignificant (for details, see Materials and Methods).

In another type of experiment, eggs were lysed in suspension, giving rise to large Triton-treated cortical fragments (Fig. 3 *b*). These fragments, isolated by low-speed centrifugation (P3; Fig. 2), contained ~20% of the actin of the egg (values ranged from 12 to 27% in five experiments, with an average value of 19%). The presence of ≥5 mM EGTA in the isolation solution was essential to recover the actin in the P3 fraction (Fig. 3 *a*).

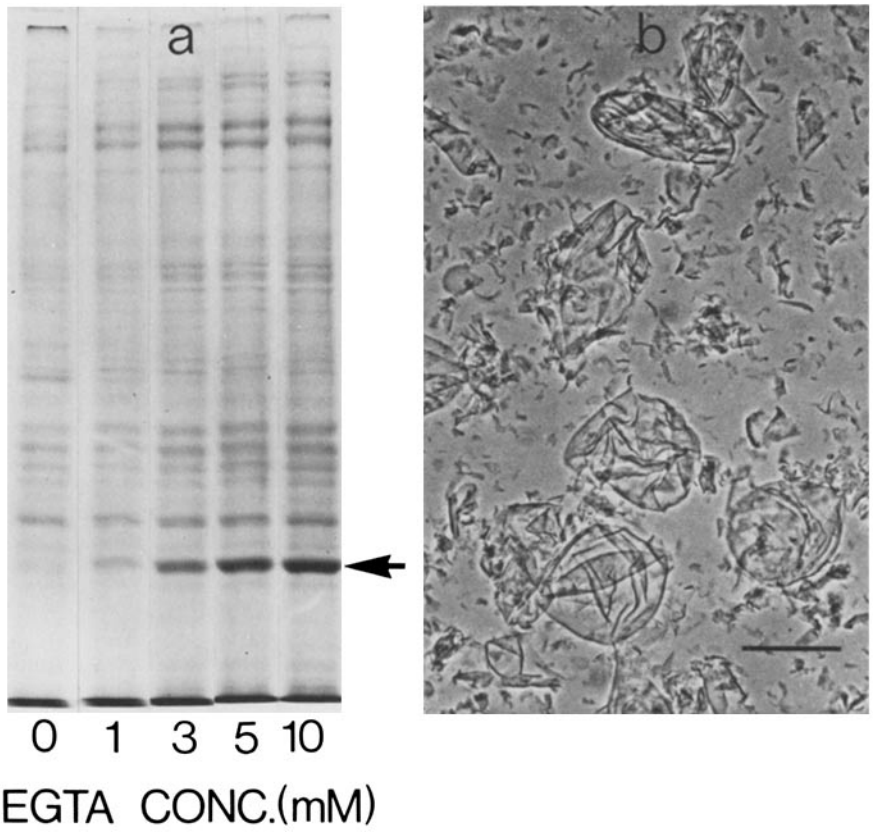

FIGURE 3 (*a*) SDS-polyacrylamide gels of unfertilized egg Triton-treated cortical fragments (P3) isolated in suspension in different concentrations of EGTA. The arrow shows where purified skeletal muscle actin runs on identical gels. The separating gel was 7.5% acrylamide. (*b*) Light microscope photograph of Triton-treated cortical fragments (P3) prepared in the presence of 5 mM EGTA. Bar, 100 μm. × 130.

Quantitation of Actin on Triton-Treated Cortical Fragments as a Function of Time of Development after Fertilization

Eggs were fertilized and allowed to develop in filtered sea water as a monolayer adhering to protamine sulfate-coated petri dishes. Development through first cleavage proceeded very synchronously under these conditions (Fig. 4); development was followed through blastula stage and appeared normal by light microscopy. The amount of actin per square micron of apparent surface area of Triton-lysed eggs was found to be between 1.5×10^{-9} and 4.0×10^{-9} μg throughout the first cell cycle.

Electron Microscopy of Triton-Treated Cortical Fragments of Eggs before and after Fertilization

Previous investigators have noted difficulty in observing significant numbers of microfilaments in the cortex of unfertilized eggs (3, 28), whereas filaments and filament bundles are prevalent in fertilized egg cortices (3, 4, 7, 8, 15). Since our experiments indicated that a significant amount of actin is present in our Triton-treated cortical preparations of unfertilized eggs, we examined these cortical fragments for actin filaments using techniques described previously (6, 8, 9, 53). Although filamentous structures ~70 Å wide were sometimes apparent (see arrow, Fig. 6), the general appearance of the Triton-treated cortical fragments from unfertilized eggs was that of amorphous material concentrated in hexagonally-packed patches 0.2–0.3 μm in diameter (Figs. 5 and 6).

In contrast to unfertilized eggs, 70 Å filaments were very prevalent on Triton-treated cortical fragments of fertilized eggs (Figs. 7 and 8), as already reported by Burgess and Schroeder (8). At 5 min after fertilization, many filaments were ap-

parent (Fig. 7), and later in the first cell cycle microvillar filament bundles were abundant (Fig. 8).

Biochemical and Structural Properties of Actin from Triton-Treated Cortical Fragments of Unfertilized Eggs

Only a single species of actin was apparent when the low-speed pellet fraction (P3) of unfertilized eggs was examined by isoelectric focusing; the actin in P3 comigrated with β-actin from chick embryo fibroblasts (Fig. 9).

Approx. 75% of the actin was solubilized by dialysis of P3 against G-buffer. This solubilized actin (S4; see Fig. 2) was ~50% pure, as judged by PAGE (Fig. 10). The actin from S4 was purified to near homogeneity (Fig. 10) by Sephadex G-150 filtration followed by DEAE-cellulose chromatography (see Materials and Methods). The purified preparation is referred to as cortical actin.

The cortical actin formed 70 Å filaments in 0.1 M KCl and 3 mM $MgCl_2$ (Fig. 11 *b*). In contrast, the S4 actin preparation generated amorphous aggregates under the same conditions (Fig. 11 *a*). The inability to observe actin filaments in the S4 preparation may be related to the inability to observe filaments in the cortical fragments of unfertilized eggs (Figs. 5 and 6) (see Discussion).

The cortical actin formed Mg^{2+}-paracrystals upon addition of $MgCl_2$ to 50 mM (Fig. 12 *a*). Optical diffraction patterns of such paracrystals (Fig. 12 *b*) were characteristic of those obtained for purified actin from muscle (46) and from *Dictyostelium discoideum* (51).

An important property of actin is its ability to interact with myosin in a very specific way. This interaction can be quantitated by measuring activation of myosin ATPase activity. The sea urchin cortical actin was nearly as efficient as *Dictyostelium* actin in activating *Dictyostelium* myosin ATPase. From Fig. 13 it is apparent that 0.3 μg actin/μg myosin is required for half-maximal activation of the myosin ATPase. In analogous experiments using *Dictyostelium* actin, the value for half-maximal activation was 0.2–0.3 μg actin/μg myosin (Kuczmarski and Spudich, unpublished observations).

DISCUSSION

We have isolated Triton-treated cortical fragments of unfertilized sea urchin eggs and have studied the actin associated with them. The amount of actin associated with these cortical fragments was determined using polycation-coated petri dishes to isolate them. The technology used was similar to that devised by Vacquier (53) to study cortical granules of sea urchin eggs and adapted by Clarke et al. (9) to examine actin filaments on the cytoplasmic surface of *Dictyostelium* amoebae. Treatment of cells with Triton X-100 to expose the actin associated with the cytoplasmic surface was described by Brown et al. (6). These techniques have already proven very useful to study actin associated with sea urchin egg membranes. Thus, Burgess and Schroeder (8) showed that microvilli of fertilized sea urchin eggs contain actin filaments of uniform polarity. Furthermore, Burgess (7) reported that acrylamide gel electrophoresis of fertilized egg cortices isolated on polycation-coated surfaces reveals a predominant component with the same electrophoretic mobility as muscle actin. This component was relatively minor in his preparations of unfertilized egg cortices.

In our cortical preparations of unfertilized eggs, we find a considerable amount of actin. Assuming that the cortical fragment remaining on the petri dish is representative of that of the entire egg surface, we calculate that ~20% of the total egg actin is associated with the cortical layer of the unfertilized egg under the conditions of our experiments. Our assumption that no extensive asym-

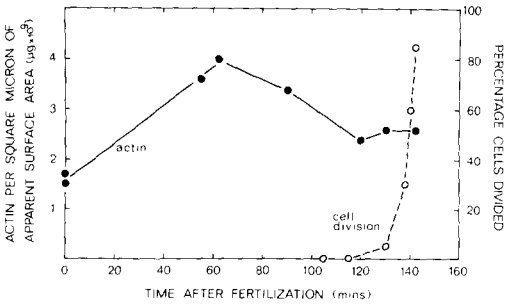

FIGURE 4 The amount of actin on the Triton-treated cortical fragments as a function of time after fertilization. Cortical fragments of eggs attached to polycation-coated petri dishes were prepared as described in Materials and Methods. Values for actin per square micron of apparent surface area for unfertilized eggs are plotted at zero time. No values were obtained for actin per square micron of apparent surface area for fertilized eggs earlier than 50 min after fertilization because of the time required to remove sperm from the fertilized egg suspension. Percentage cells divided was determined by counting the percentage of eggs that had a nearly complete cleavage furrow. The eggs were developed at 15°C.

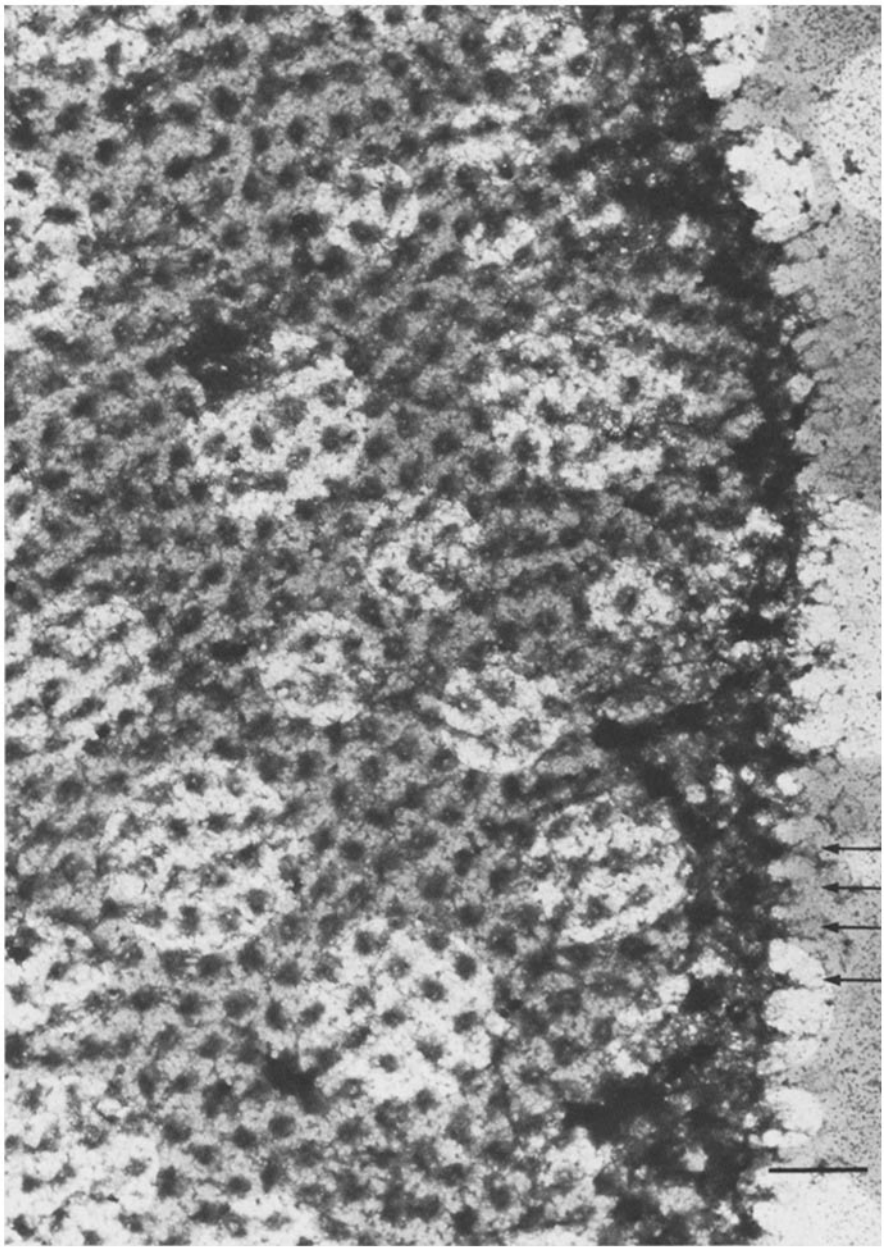

FIGURE 5 Electron micrograph of a portion of a Triton-treated cortical fragment of an unfertilized egg. The method of preparation is given in Materials and Methods. What may be Triton-extracted remnants of short papillae of unfertilized eggs are visible at the edge on the right of the cortical fragment (e.g., see arrows). The dense hexagonally-packed patches of material probably represent papillae viewed from a different angle. The large light circles are holes in the carbon coating layer of the grid. Bar, 1 μm. × 13,000.

metric redistribution of actin results from attachment of an egg to the polycation-coated surface is supported by the finding that ~20% of the egg actin is associated with the large cortical fragments obtained by lysing eggs in suspension. Thus, we estimate that an unfertilized egg has $\sim 2 \times 10^{-9}$ μg actin/μm^2 apparent surface area, or $\sim 4 \times 10^{-5}$ μg actin on the total inner surface of a single

unfertilized egg (the total apparent surface area of an *S. purpuratus* egg is $\sim 2 \times 10^4$ μm^2, using 80 μm as the diameter of an egg). This amount of actin if distributed uniformly would cover about one-half of the apparent surface area of the egg as a monolayer. Furthermore, the amount of actin in the cortical preparations of the unfertilized egg could account for nearly all of the actin filaments needed to fill the microvilli after fertilization, using 1.8 μm as the average length of the microvilli after the second burst of elongation (40).[4]

The increase in the amount of actin per square micron of apparent surface area after fertilization (see Fig. 4) may be significant since this trend occurred in other similar experiments. The significance of the increase, however, is difficult to assess. It may represent a further recruitment of soluble, cytoplasmic actin into the cortical layer. Alternatively, the apparent increase could reflect some asymmetric distribution of the actin associated with the cortex; if the actin is not uniformly distributed on the fertilized egg surface, we could have selected for a particular portion of the fertilized cortical layer that was enriched for actin. It is interesting to note that these eggs which developed as a monolayer attached to protamine sulfate-coated petri dishes always had their first-cleavage planes oriented perpendicular to the surface of the dish.

Since much of the actin in the unfertilized egg Triton-treated cortical fragments does not appear to be filamentous, it is tempting to speculate that G-actin is sequestered on the inner surface of the unfertilized egg plasma membrane, perhaps in the dense patches apparent in Figs. 5 and 6, and is induced to polymerize upon fertilization, giving rise to microvilli. Thus, this situation appears analogous to that described by Tilney (48) for the acrosomal reaction in *Thyone* sperm. Indeed, the polarity of the actin filaments in the egg microvilli (4, 8) is the same as that described by Tilney (48) for the sperm acrosomal process. Although the data presented in our report are consistent with the concept that most of the actin on the unfertilized egg membrane is nonfilamentous, we have not ruled out the possibility that some of it may indeed be in a filamentous state. For example, each papilla (12) could contain an actomere-like structure similar to that which exists in *Thyone* sperm heads (49). Such a structure may be difficult to visualize by the techniques of electron microscopy that we have employed here. However, other investigators using other techniques have also failed to observe notable amounts of actin filaments on unfertilized eggs (3, 28). Furthermore, the inability to make actin filaments from our S4 preparations, which are derived from the Triton-treated cortical preparations and are impure, argues for the presence of some inhibitor of actin filament formation. Indeed, actin purified from the S4 preparations polymerizes readily. Thus, G-actin may be sequestered on the inner surface of the unfertilized egg plasma membrane in a form that is unable to polymerize, and fertilization may induce actin polymerization.

If actin that is associated with the egg surface is induced to form filaments when the egg is fertilized, what could be the biochemical signal for this transformation? Fertilization results in an increase in the intracellular pH of the egg from ~6.4 to ~7.3 (20, 24, 42). Recently, Begg and Rebhun (3) reported that actin on isolated cortical fragments of unfertilized eggs will polymerize when the pH is raised from 6.5 to 7.5. A pH-dependent transformation of actin from a nonpolymerized to a filamentous form has been described by Tilney et al. (50) for the acrosomal reaction of echinoderm sperm. Our buffer is similar to that of Begg and Rebhun (3) except that we include Triton X-100 and prepare Triton-treated cortical fragments of the eggs at pH 6.8. We then solubilize the actin by dialysis against a low ionic strength buffer (S4 preparation). This solubilized actin preparation does not form proper filaments in a buffer containing 0.1 M KCl and 3 mM $MgCl_2$ at pH 7.5. Therefore, the effect of pH on actin assembly described by Begg and Rebhun (3) would not appear to act directly on the actin. The presence of Triton X-100 in our lysis buffer may remove or destroy a H^+-sensitive component in the cortical fragments. Thus, actin in the unfertilized egg may be maintained in a nonfilamentous form in vivo

[4] Since actin has a mol wt of 4.2×10^4, 4×10^{-5} μg actin in the cortical layer of an unfertilized egg equals 5.7×10^8 molecules. Assuming that actin is a sphere of 50 Å diameter, 5.7×10^8 molecules of actin close-packed in two dimensions would occupy about 1.1×10^4 μm^2, about one-half of the total apparent surface area of the egg. If this amount of actin were equally distributed amongst the 1.2×10^5 microvilli present on the fertilized egg (41), there would be 4.8×10^3 molecules of actin per microvillus. Since there are ~7 actin filaments per microvillus (8), each would have 6.8×10^2 molecules and be ~1.9 μm long (there are 28 Å per actin monomer along an actin filament; see reference 44).

by some inhibitor of polymerization. Relief of this inhibition may involve a change in another component (or organelle) induced by an increase in pH.

Our results suggest that Ca^{2+} can affect the Triton-treated cortical preparations in some way since the amount of actin associated with the cortical fragments that we isolate in the absence of

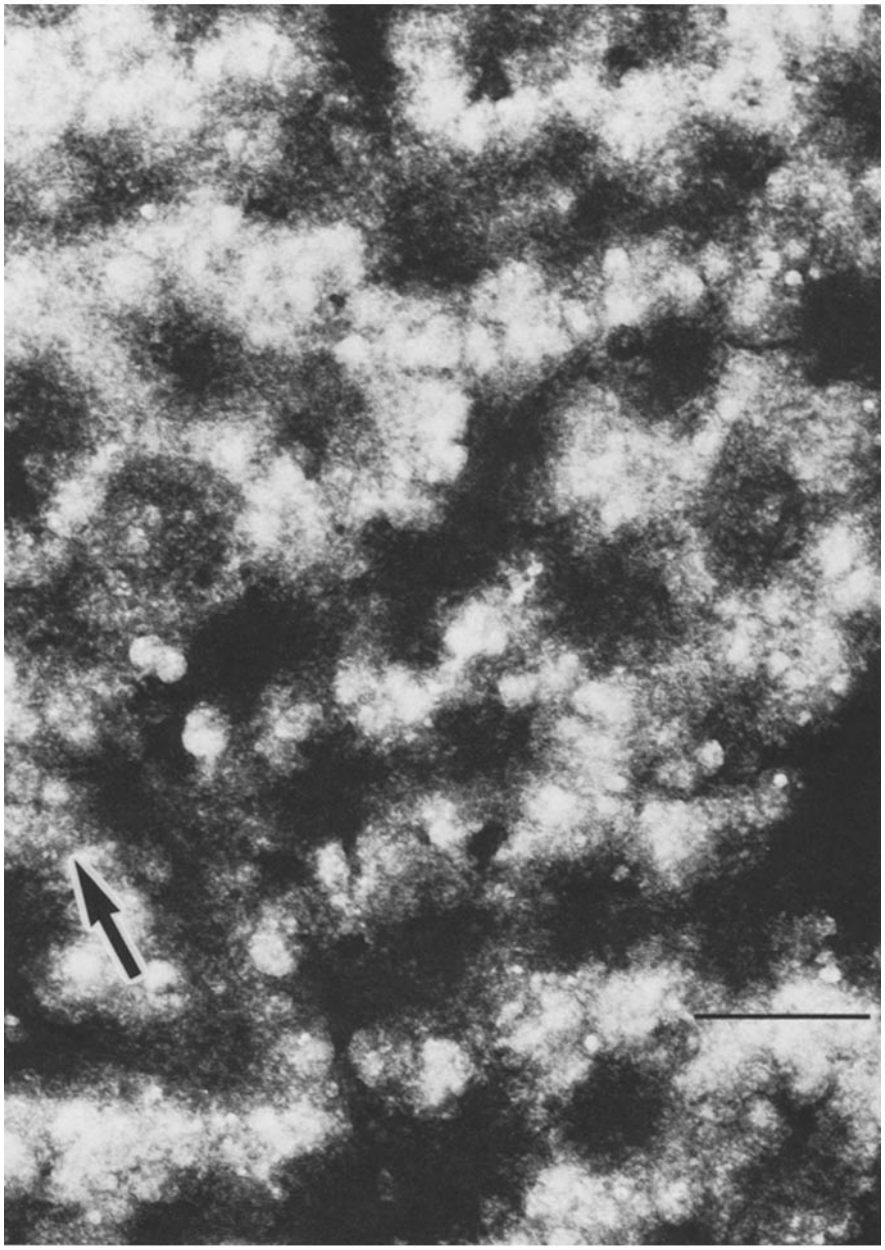

FIGURE 6 High magnification view of a portion of a Triton-treated cortical fragment of an unfertilized egg. The arrow indicates where there may be filament bundles running between the dense patches. Bar, 0.4 μm. × 58,000.

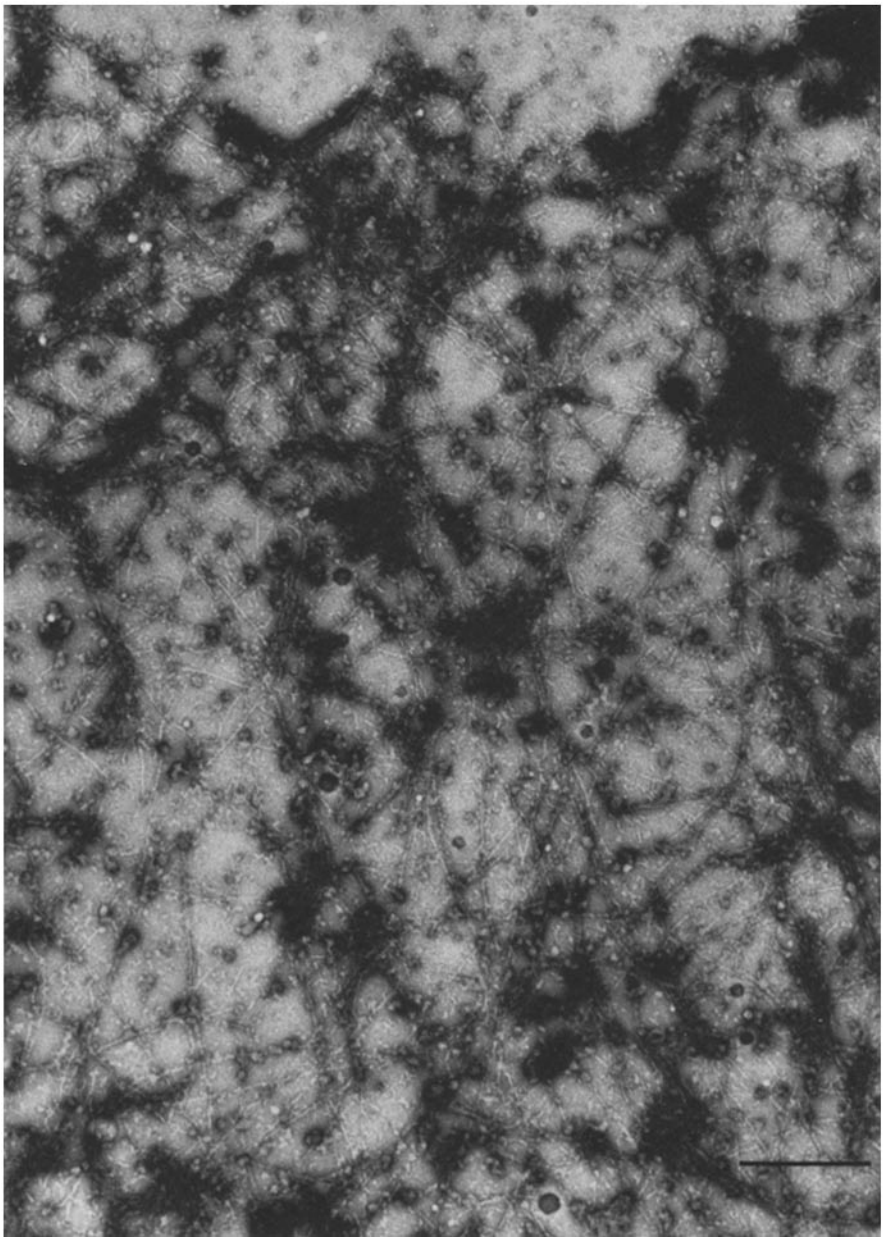

FIGURE 7 Electron micrograph of a portion of a Triton-treated cortical fragment of an egg 5 min after fertilization. The cortical fragment was prepared as those shown in Figs. 5 and 6. Large numbers of 70 Å filaments and some small filament bundles are visible. Other areas often showed small filament bundles to be more prevalent. The edge of the cortical fragment is apparent at the top. Bar, 0.5 μm. × 35,000.

EGTA is very small. This Ca^{2+} effect, like the pH effect discussed above, appears to be indirect since addition of Ca^{2+} to cortical fragments isolated in the presence of EGTA does not cause release of the actin (A. Spudich and J. A. Spudich, unpublished observations).

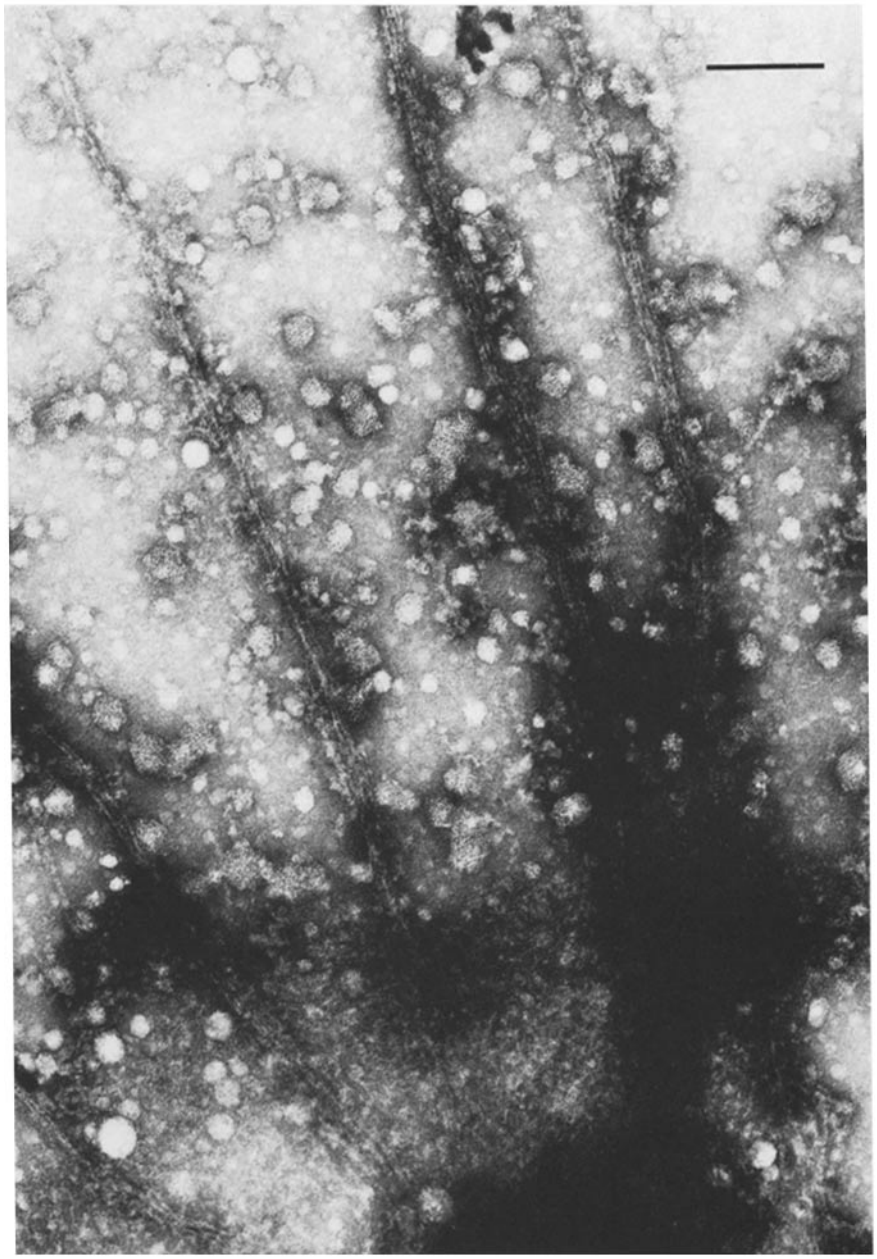

FIGURE 8 Electron micrograph of a portion of a Triton-treated cortical fragment of an egg 2 h after fertilization. The cortical fragment was prepared as those shown in Figs. 5–7. Bundles of microfilaments are seen in elongated microvilli at the edge of the cortical fragment. Some of these filament bundles show a 130 Å periodicity. Bar, 0.2 μm. × 77,000.

The possibility that nonfilamentous actin may be associated with cell surfaces and primed for rapid polymerization may not be restricted to such specialized cells as eggs and sperm. For example, ~15% of actin in *Dictyostelium* amoebae is associated with isolated membranes and remains as an

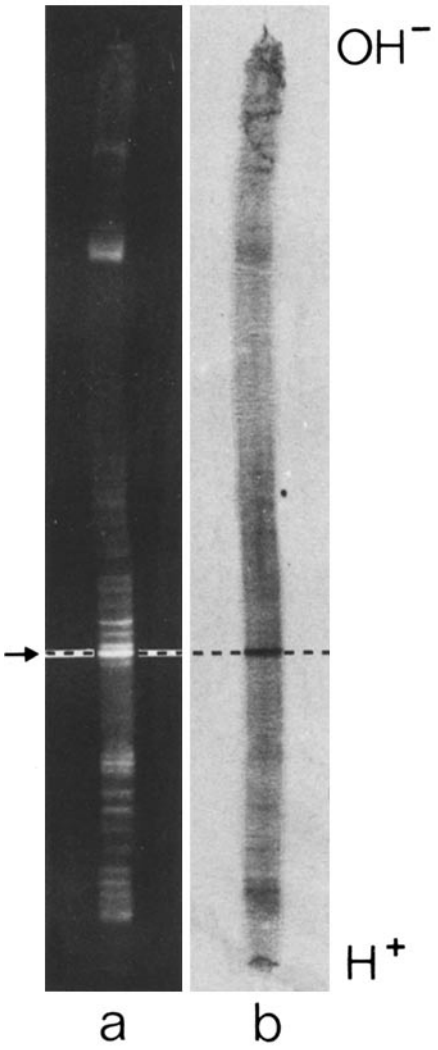

FIGURE 9 Isoelectric focusing gel of unfertilized egg Triton-treated cortical fragments (P3) isolated in suspension. To a sample of P3 containing 3 μg of protein, an aliquot of [^{35}S]methionine-labeled chick embryo fibroblast extract (35) was added. The isoelectric focusing gel was run as described by O'Farrell (33) with minor modifications (35). It was then dried flat and (*a*) autoradiographed to show that the (*b*) Coomassie Blue-stained actin band from the P3 preparation comigrates with the major radioactive band of the labeled fibroblast extract (β-actin; see arrow and reference 35). This volume of fibroblast extract alone on an isoelectric focusing gel did not give a Coomassie-stained band (data not shown).

insoluble cytoskeletal residue when the membrane fraction is extracted with Triton X-100 (43, 45). In related experiments, *Dictyostelium* amoebae were attached to a surface coated with polylysine, and the upper portion of the cells was sheared off with a stream of buffer (9). In such experiments, actin filaments were not readily apparent on the cytoplasmic side of the cell surface when cells were broken open immediately after attachment to the substratum. When cells were broken open ~1 min after attachment, filaments were apparent. Thus, there may be a contact-stimulated transformation of actin into a filamentous form, analogous to that postulated for the eggs and sperm. Further work is necessary to determine to what extent such a cell surface associated transformation of actin assembly is a general phenomenon.

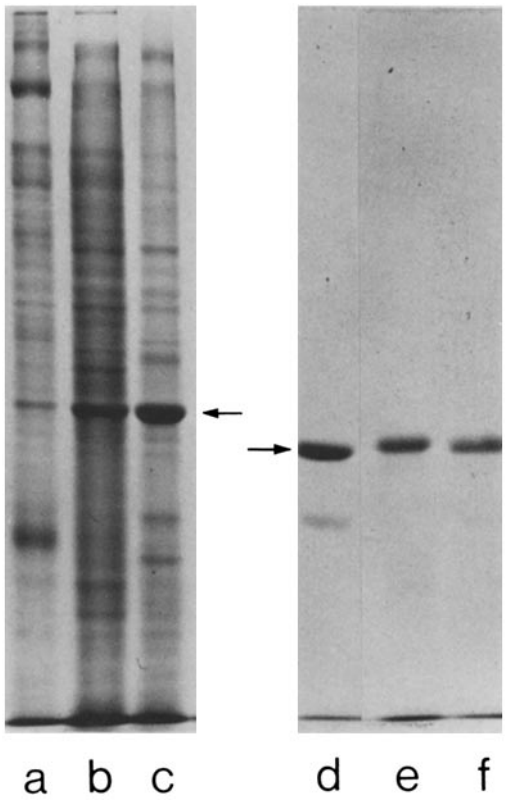

FIGURE 10 SDS-polyacrylamide gels of fractions obtained in the course of purification of unfertilized egg cortical actin (see flow diagram, Fig. 2). (*a*) lysed eggs, 4 μg protein; (*b*) P3, 10 μg; (*c*) S4, 7 μg; (*d*) egg cortical actin, 2.5 μg; (*e*) *Dictyostelium* actin, prepared according to Uyemura et al. (51), 1 μg; (*f*) egg cortical actin plus *Dictyostelium* actin, 0.5 μg each. The gels contained (*a-c*) 8.5% acrylamide and (*d-f*) 8.0% acrylamide. The arrows show where purified skeletal muscle actin runs on identical gels.

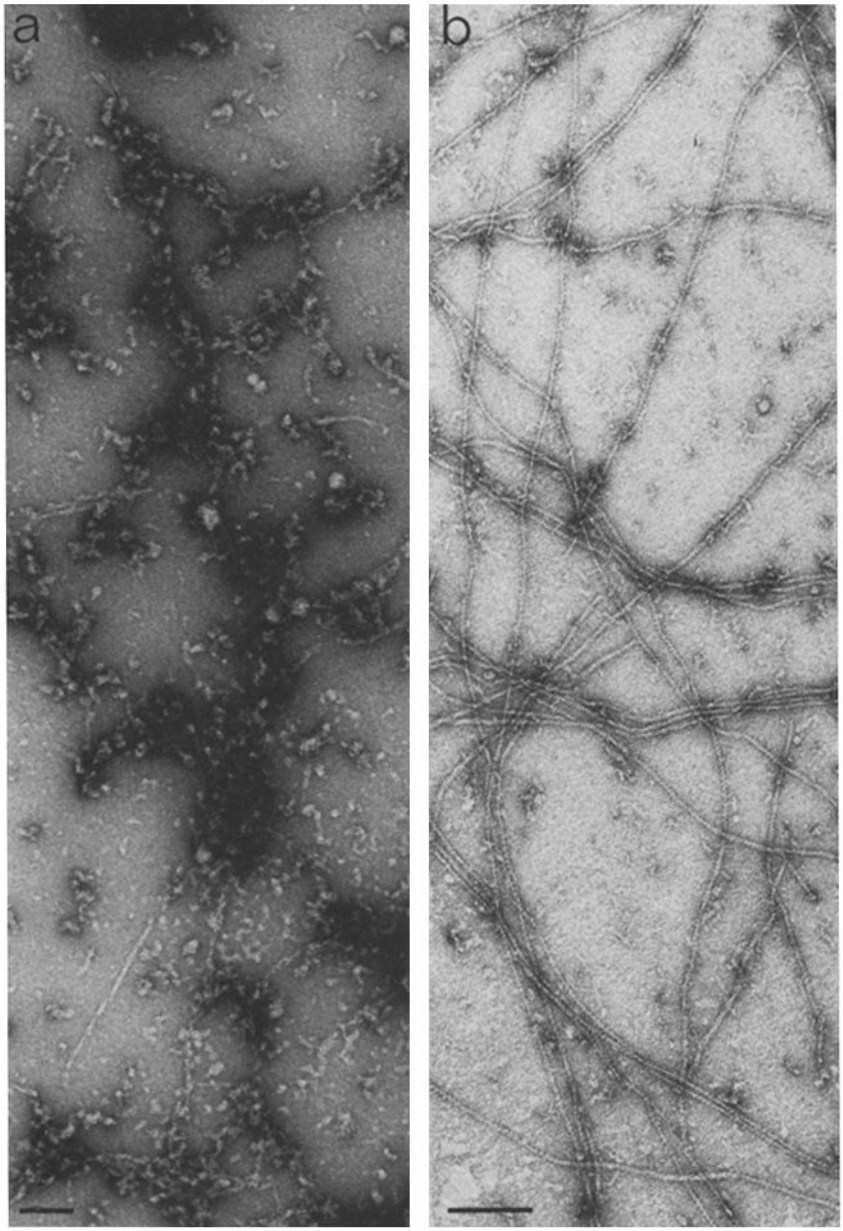

FIGURE 11 Electron micrographs of (*a*) an S4 preparation, and (*b*) purified cortical actin from unfertilized eggs. Both preparations (each ~0.5 mg/ml in actin) were induced to polymerize by adjusting the concentrations of $MgCl_2$ to 3 mM and KCl to 0.1 M, and were then negatively stained with 1% uranyl acetate. (*a*) Bar, 0.1 μm; × 71,000. (*b*) Bar, 0.1 μm; × 110,000.

It is our pleasure to thank Dr. Dennis O. Willows for providing facilities at the University of Washington Friday Harbor Laboratories where this work was initiated in the summer of 1975 and continued in the summer of 1976. We are greatly indebted to Drs. Joseph Bryan, Leslie Wilson and T. E. Schroeder for teaching us how to work with sea urchin eggs and for numerous discussions. We also wish to thank Drs. Victor Vacquier and David Epel for stimulating and encouraging discussions and for sharing with us much information on unfertilized and fertilized eggs.

This work was supported by grants to J. A. Spudich

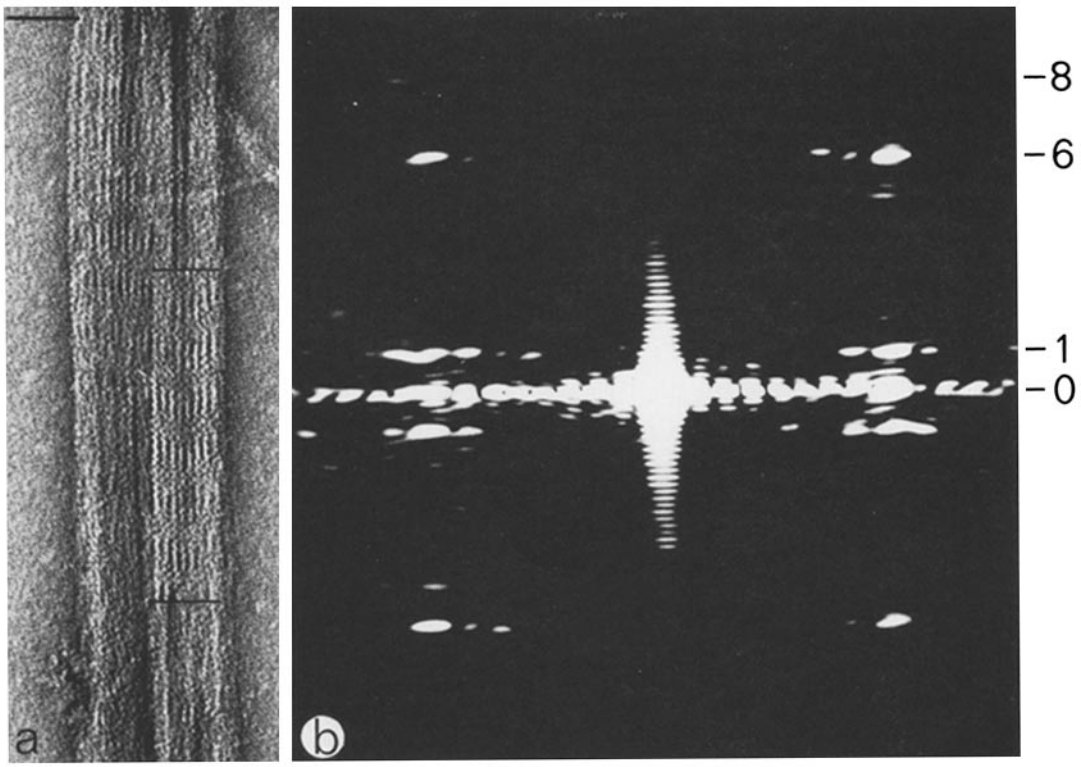

FIGURE 12 (*a*) Mg^{2+}-paracrystal of purified cortical actin, and (*b*) an optical diffraction pattern of the Mg^{2+}-paracrystal. The paracrystal was formed by making the actin solution 50 mM in $MgCl_2$. The optical diffraction pattern is of the area between the lines shown in the micrograph in *a*. The numbers 0, 1, 6, and 8 refer to layer lines in the diffraction pattern. (*a*) Bar, 0.05 μm; × 194,000.

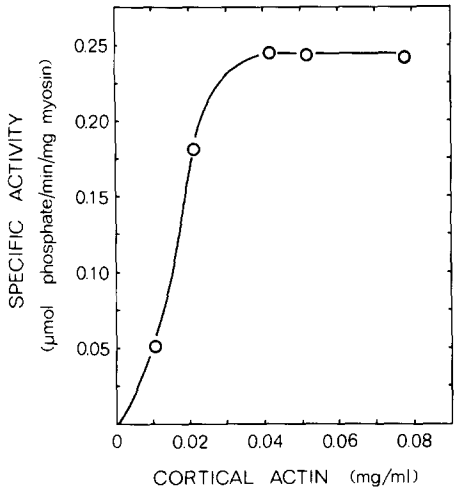

FIGURE 13 Activation of myosin ATPase by purified cortical actin from unfertilized eggs. Preparation of *Dictyostelium* myosin and the assay for ATPase activity were carried out as described by Mockrin and Spudich (32). Each reaction mixture (0.1 ml final vol) contained 6 μg of myosin and the amounts of cortical actin shown.

from the American Cancer Society (VC121) and the National Institutes of Health (GM25240).

Received for publication 4 December 1978, and in revised form 26 February 1979.

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