Structural Organization of Actin in the Sea Urchin Egg Cortex: Microvillar Elongation in the Absence of Actin Filament Bundle Formation

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ABSTRACT We have investigated the relationship between the formation of actin filament bundles and the elongation of microvilli (MV) after fertilization in sea urchin eggs. In a previous study (1979, *J. Cell Biol.* 83:241-248) we demonstrated that increased pH induced the formation of actin filaments in isolated sea urchin egg cortices with the concomitant elongation of MV. On the basis of these results we suggested that increased cytoplasmic pH after fertilization causes a reorganization of cortical actin, which in turn provides the force for MV elongation. To test this hypothesis, we compared the morphology of microvilli in eggs activated with and without the release of fertilization acid. Activation of eggs in normal sea water with the calcium ionophore A23187 causes the release of fertilization acid and the elongation of MV containing core bundles of actin filaments. Eggs activated with A23187 in Na^+-free water do not undergo normal fertilization acid release but develop elongated, flaccid MV. These MV contain an irregular network of actin filaments rather than the parallel bundles of filaments found in normal MV. The addition of 40 mM NaCl to these eggs results in the release of H^+ and the concomitant conversion of flaccid MV to erect MV containing typical core bundles of actin filaments. Identical results are obtained when 10 mM NH_4Cl is substituted for NaCl. The induction of cytoplasmic alkalinization in unactivated eggs with NH_4Cl does not cause either MV elongation or the formation of actin filament bundles. These results suggest that: (*a*) the elongation of MV is stimulated by a rise in intracellular free Ca^{++} concentration; (*b*) actin filament bundle formation is triggered by an increase in cytoplasmic pH; and (*c*) the formation of actin filament bundles is not necessary for MV elongation but is required to provide rigid support for MV.

Fertilization of the sea urchin egg leads to a series of ionic changes which initiate a variety of developmental events. Within the first few seconds after fertilization, an influx of Na^+ results in the depolarization of the egg plasma membrane and the establishment of the fast block to polyspermy (22, 23, 37). Membrane depolarization is rapidly followed by a transient increase in intracellular free Ca^{++} concentration which induces the "early events" of fertilization, including cortical granule exocytosis, elevation of the fertilization envelope, and the activation of NAD kinase (see references 16 and 17 for reviews). Beginning ~1 min after fertilization, an efflux of protons from the egg results in an increase in cytoplasmic pH (24, 25, 31, 32). This alkalinization of the egg's cytoplasm triggers the "late events" of fertilization such as the development of K^+-conductance (33, 37), activation of amino acid transport (15), polyadenylation of mRNA (41), and acceleration of the rate of protein synthesis (18, 19).

During the period in which these ionic changes take place, a dramatic reorganization occurs in the structure of the egg's surface. Insertion of the cortical granule membrane into the egg plasma membrane during exocytosis results in the formation of a mosaic membrane (12, 14, 30). At the same time, the numerous short microvilli that cover the surface of the unfertilized egg elongate (14, 29, 30, 34), a process which may be

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responsible for taking up the excess membrane introduced by the cortical granules (13, 30). These elongated microvilli contain core bundles of actin filaments which resemble those from the microvilli of intestinal epithelial cells (1, 3, 5, 20). The temporal relationship between microvillar elongation and cytoplasmic alkalinization suggested to us that, as in the formation of the acrosomal process in echinoderm sperm (40), the increase in cytoplasmic pH might induce the polymerization of cortical actin, which in turn would provide the force for microvillar elongation. In a previous paper (1), we reported that the state of actin in cortices isolated from unfertilized *Strongylocentrotus purpuratus* eggs depends upon the pH of the isolation medium: cortices which are isolated at the pH of the unfertilized egg (6.5–6.7) do not contain filamentous actin, while those isolated at the pH of the fertilized egg (7.3–7.5) contain elongated microvilli and organized arrays of actin filaments. These results strongly suggest that cytoplasmic pH regulates the assembly of cortical actin filaments and the concomitant elongation of microvilli at fertilization.

To test this hypothesis we compared the morphology of microvilli on intact eggs activated with and without the release of fertilization acid. Johnson et al. (25) have demonstrated that Na^+ is required for acid release at fertilization. In sea water (SW) in which NaCl is replaced by choline chloride, acid release does not occur upon activation of the egg. The subsequent addition of Na^+ to the SW initiates the release of H^+, with the rate of release being proportional to the Na^+ concentration (25). Since fertilization is inhibited in Na^+-free SW (9), we used the calcium ionophore A23187 to parthenogenetically activate eggs.

The results reported here demonstrate that the in vivo control of cortical actin filament formation and microvillar elongation in sea urchin eggs is more complex than we originally proposed and appears to involve the cytoplasmic free Ca^{++} concentration as well as pH. A preliminary report of these results has appeared previously (2). Similar results have also been reported by Carron and Longo (6).

MATERIALS AND METHODS

Three species of sea urchins were used for these studies: *S. purpuratus, Lytechinus pictus*, and *Arbacia punctulata. S. purpuratus* and *L. pictus* were purchased from Pacific Biomarine Co. (Venice, CA) and *A. punctulata* were obtained from either the Marine Biological Laboratory (MBL) (Woods Hole, MA) or G. W. Nobel (Panacea, FL). Females were induced to shed their eggs by injecting 0.5 M KCl into the coelomic cavity. Testes were dissected out and stored "dry" at 4°C. Eggs were washed once in Ca^{++}/Mg^{++}-free artificial sea water (ASW) to remove the jelly coat (1) and were then washed three times in ASW (MBL formula in reference 7).

Eggs either were fertilized by the addition of two to three drops of a concentrated sperm suspension or were parthenogenetically activated with the Ca^{++} ionophore A23187 (11, 35). The ionophore was stored as a 1 mg/ml stock in domethylsulfoxide and was used at a final concentration of 20 μM for egg activation. Eggs of *S. purpuratus* and *L. pictus* were maintained at 16–18°C and those of *A. punctulata* at 20–24°C.

For scanning electron microscope (EM) studies, fertilization envelopes were removed after activation by suspending eggs in 1 M urea containing 2 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid), pH 8.0, for 4 min. Demembranated eggs were washed three times in Ca^{++}-free ASW (5) containing 20 mM HEPES and 1 mM EGTA, pH 8.0, and were incubated in the same solution as a 0.1–0.5% suspension with constant stirring.

Identical results were obtained with all three species of sea urchins used in these studies. However, the transmission electron micrographs of intact eggs are restricted to *Arbacia* since optimal actin fixation was obtained with this species.

Activation of Eggs in Choline-substituted SW

To prevent the release of acid by eggs upon activation, they were treated with A23187 in ASW in which NaCl was replaced with choline chloride (25). Choline chloride was purchased from Sigma Chemical Co. (St. Louis, MO) and was further purified by recrystallization from hot ethanol. Choline-substituted ASW was composed of: 424 mM choline chloride, 26 mM $MgSO_4 \cdot 7H_2O$, 23 mM $MgCl_2 \cdot 6H_2O$, 12 mM $CaCl_2 \cdot 2H_2O$, 9 mM KCl, and 2 mM $KHCO_3$. The pH was adjusted to 7.8–8.0 with KOH. Eggs were washed three times in choline-SW to remove traces of Na^+ and activated with A23187 as described above. After activation, acid release was induced by the addition of 40 mM NaCl to the egg suspension. This concentration of NaCl was chosen because it produced the maximum rate of H^+ efflux (25).

Measurement of Acid Release

The release of acid from eggs was measured after normal fertilization in ASW and after activation with A23187 in both ASW and choline-SW. A 2% suspension of eggs (2-ml packed egg volume in 100 ml of SW) was gently stirred with a magnetic stirrer, and the pH of the SW was monitored with a Corning Model 130 pH meter. The output of the pH meter was displayed as a continuous recording on an Altex 210 chart recorder.

Alteration of Cytoplasmic pH with NH_4Cl and Sodium Acetate

Unfertilized eggs were treated with 10 mM NH_4Cl in ASW for 30 min to induce cytoplasmic alkalinization (18, 36). Cytoplasmic acidification was accomplished by incubating fertilized eggs in 10 mM sodium acetate for 30 min (19).

Cortex Isolation and Myosin Subfragment-1 Decoration

Cortices were isolated by the method of Begg and Rebhun (1) from eggs which had been activated with A23187 in either ASW or choline-SW. The pH of the cortex isolation medium was adjusted to 6.5 with KOH rather than NaOH to prevent the Na^+-dependent release of H^+ from eggs activated in Na^+-free medium. Isolated cortices were decorated with myosin subfragment-1 (S-1) in cortex isolation medium by the method of Begg et al. (3).

Electron Microscopy

Eggs were fixed for transmission EM in 1% glutaraldehyde (8% stock; EMS, Inc., Fort Washington, PA), 1% paraformaldehyde in Ca^{++}-free ASW, pH 7.2–7.4 for 45 min at 15–18°C. After fixation, eggs were washed three times in Ca^{++}-free ASW, pH 7.4, and postfixed in 0.5% OsO_4 in 0.1 M sodium phosphate buffer, pH 6.0, for 30 min on ice. They were subsequently washed three times in deionized H_2O and incubated for 45 min at room temperature in unbuffered aqueous 1% uranyl acetate before being dehydrated with ethanol through a graded series and embedded in Epon/Araldite. Pellets of isolated cortices were fixed as described by Begg and Rebhun (1). Thin sections were stained with uranyl acetate and lead citrate and were examined in a Philips 300 electron microscope operated at an accelerating voltage of 60 kv. The microscope was calibrated with a 54,800 lines/inch replica grating.

Eggs were processed for scanning EM as described above, except that the eggs were allowed to settle onto poly-L-lysine-coated cover slips after aldehyde fixation. Samples were dehydrated through a graded series of ethanol and critical-point dried from CO_2. The egg-containing coverslips were attached to stubbs with colloidal silver paste and coated with gold-palladium. Samples were examined in an ETEK Auto-Scan Scanning Electron Microscope at an accelerating voltage of 20 kv.

RESULTS

Ionophore Activation in Na^+-containing SW

Parthenogenetic activation of sea urchin eggs with the Ca^{++} ionophore A23187 results in a transient increase in the intracellular free Ca^{++} concentration (42) and the release of fertilization acid into the surrounding SW (28). In our hands, activation of a 2% suspension of eggs in ASW results in a decrease in the pH of the SW of ~0.4 pH unit. This value is slightly greater than that observed when the same concentration of eggs is activated with sperm (0.35 pH unit).

The concentration of A23187 required to give 100% activation varies somewhat between different batches of eggs (10–20 μM). 20 μM A23187 was used routinely in all experiments

reported here. At concentrations of ionophore greater than that required for complete activation, the amount of acid released is independent of ionophore concentration, indicating that acid efflux is not due to the direct pumping of H^+ out of the egg by the ionophore (results not shown).

Ionophore-activated eggs undergo normal cortical granule exocytosis, fertilization membrane elevation, and microvillar elongation (10, 11, 35). As in fertilized eggs (1, 7), these elongated microvilli contain core bundles of actin filaments (Figs. 3 *a*, 4 *a*, and 5 *b*).

Ionophore Activation in Na^+-free SW

Activation of a 2% suspension of eggs in Na^+-free ASW with A23187 results in the immediate release of a small amount of acid, equivalent to ~25% of the normal acid release measured in Na^+-containing medium (Fig. 1). This Na^+-independent acidification has been shown to be due to the release of acidic cortical granule contents (16) and does not reflect an alkalinization of the cytoplasm (32). After the initial phase of acid release the pH of the egg suspension remains constant until Na^+ is added (Fig. 1). The addition of 40 mM NaCl to the egg suspension elicits the remainder of the acid release, with the sum of the two phases of acid release producing the normal degree of acidification observed in Na^+-containing SW. Direct measurement of intracellular pH with a micro pH electrode has demonstrated that this Na^+-induced phase of acid release results in the normal degree of cytoplasmic alkalinization observed at fertilization (32).

As described above, activation of eggs with calcium ionophore in ASW induces the formation of elongated microvilli (Fig. 2 *a*) containing core bundles of actin filaments (Fig. 3 *a*). From our previous studies of the effects of pH on the polymerization of actin in the sea urchin egg cortex we predicted that microvilli would fail to elongate in eggs activated in Na^+-free SW, where cytoplasmic alkalinization does not occur. Fig. 2 *b* shows an example of an egg activated in choline-substituted SW. Contrary to our expectations, the microvilli have elongated; however, they appear to be flaccid and have collapsed down onto the egg surface. In addition, they are irregular in shape and frequently show a bulbous protuberance at their tips. These flaccid microvilli contain an irregular network of actin filaments (Fig. 3 *b*) rather than the parallel bundles found in normal microvilli (Fig. 3 *a*). The addition of 40 mM NaCl to these eggs causes the release of H^+ and the concomitant conversion of the flaccid to rigid microvilli containing typical core bundles of actin filaments (Figs. 2 *c* and 3 *c*).

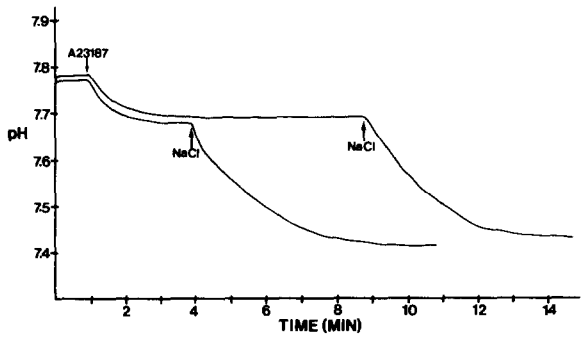

FIGURE 1 Time-course of acid release by a 2% suspension of *S. purpuratus* eggs in choline-SW. A small amount of acid release immediately follows addition of A23187, but the majority of acid release does not occur until 40 mM NaCl is added to the medium.

To determine whether the formation of filament bundles might be due to a direct Na^+ effect rather than to the Na^+-induced increase in cytoplasmic pH, we used the penetrating weak base NH_4Cl to raise the cytoplasmic pH. When 10 mM NH_4Cl is added to a suspension of ionophore-activated eggs in choline-SW, the flaccid microvilli transform into morphologically normal microvilli containing core bundles of actin filaments (Figs. 2 *d* and 3 *d*), indicating that increased cytoplasmic pH rather than Na^+ concentration causes filament bundling.

Organization of Actin in Isolated Cortices

The difference in organization of microvillar actin in eggs activated in the presence and absence of cytoplasmic alkalinization is demonstrated more clearly in isolated cortices. Fig. 4 compares the morphology of cortices isolated from eggs activated with A23187 in ASW and choline-SW. Those activated in Na^+-containing medium, where cytoplasmic alkalinization occurs, show the normal organization of cortical actin filaments (Fig. 4 *a*). Core bundles of filaments project down from the microvilli into a less highly structured filament network beneath the plasma membrane. In contrast to those isolated from eggs in Na^+-containing medium, cortices isolated from eggs which were activated in choline-SW contain microvilli with a meshwork of filaments rather than typical bundles (Fig. 4 *b*). These filaments form arrowhead complexes with myosin S-1, demonstrating that they are composed of actin (Fig. 5 *a*). However, it is difficult to determine filament polarity since only short lengths of the filaments which make up the network fall within the plane of the section. After the addition of Na^+ to these "eggs," bundles of microvillar core filaments form which exhibit the uniform, basally oriented polarity typical of the fertilized egg (Fig. 5 *b*).

Cytoplasmic Alkalinization in the Absence of a Ca^{++} Transient

In the experiments reported thus far, eggs were first subjected to a transient increase in intracellular free Ca^{++} concentration, followed by cytoplasmic alkalinization. To determine whether pH exerts a direct effect on microvillar elongation in the intact egg, we used the penetrating weak base NH_4Cl to increase the cytoplasmic pH of unfertilized eggs. Zucker et al. (42) demonstrated that NH_4Cl can induce the leakage of Ca^{++} into eggs from the external medium but does not cause the release of intracellular stores of Ca^{++}. We therefore treated eggs with NH_4Cl in both Ca^{++}-free and normal Ca^{++}-containing SW. The results were identical in both cases. Treatment of unfertilized eggs with 10 mM NH_4Cl for 30 min does not induce either microvillar elongation or the formation of actin filament bundles (Fig. 6). Grainger et al. (19) have demonstrated that this treatment is sufficient to raise the cytoplasmic pH of the unfertilized egg to that of the fertilized egg. Neither increased concentrations of NH_4Cl (20 or 40 mM) nor longer incubation times (1–2 h) cause microvillar elongation or the appearance of bundles of actin filaments.

Cytoplasmic Acidification of Fertilized Eggs

To investigate whether microvillar filament bundles can be dissociated by reduced cytoplasmic pH once they have formed, both ionophore-activated and fertilized eggs were treated with 10 mM sodium acetate in ASW for 20 min at pH 6.5. Grainger et al. (19) have shown that this treatment lowers the pH of the fertilized egg to that of the unfertilized egg. No disruption of

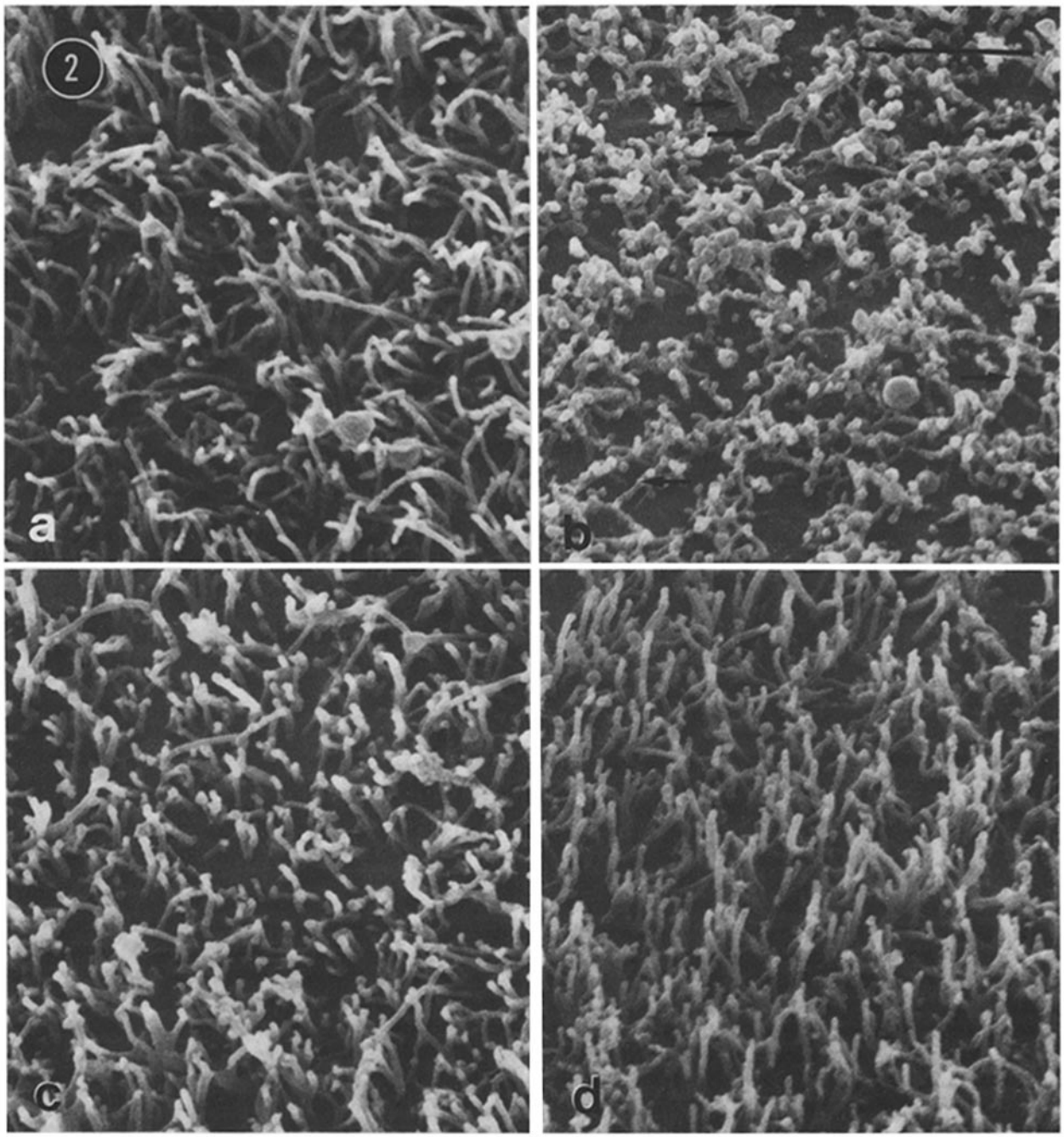

FIGURE 2 Scanning of EM of *L. pictus* eggs activated with A23187 in normal and Na^+-free ASW. (*a*) ASW. (*b*) Choline-substituted SW. Microvilli have elongated but have collapsed down on top of each other. Arrows indicate single microvilli. (*c*) Fixed 15 min. after addition of 40 mM NaCl to aliquot of egg suspension shown in *b*. (*d*) Fixed 15 min after addition of 10 mM NH_4Cl to aliquot of egg suspension shown in *b*. Bar, 2 μm. × 13,000.

microvillar core bundles was observed after sodium acetate treatment, suggesting that, once formed, these bundles are not sensitive to pH (results not shown). Previously, we reported that filament bundles in the isolated fertilized egg cortex are not dissociated by acid pH (1).

DISCUSSION

In an earlier study (1) we reported that the organizational state of actin in cortices isolated from unfertilized sea urchin eggs is determined by the pH of the isolation medium. Cortices isolated at acid pH display the normal morphology of the unfertilized egg cortex, while those isolated at basic pH develop elongated microvilli containing bundles of actin filaments. Since this result could not be duplicated by treating cortices isolated at acid pH with increased concentrations of free Ca^{++}, we concluded that pH alone controlled the organizational state of actin in the egg cortex. However, the results reported here demonstrate that in vivo the reorganization of cortical actin during microvillar elongation involves Ca^{++} as well as pH.

We do not understand why pH alone appears to control the organization of actin in the isolated egg cortex, while a combination of Ca^{++} and pH induces the reorganization of actin in the intact egg. One possibility is that the apparent absence of

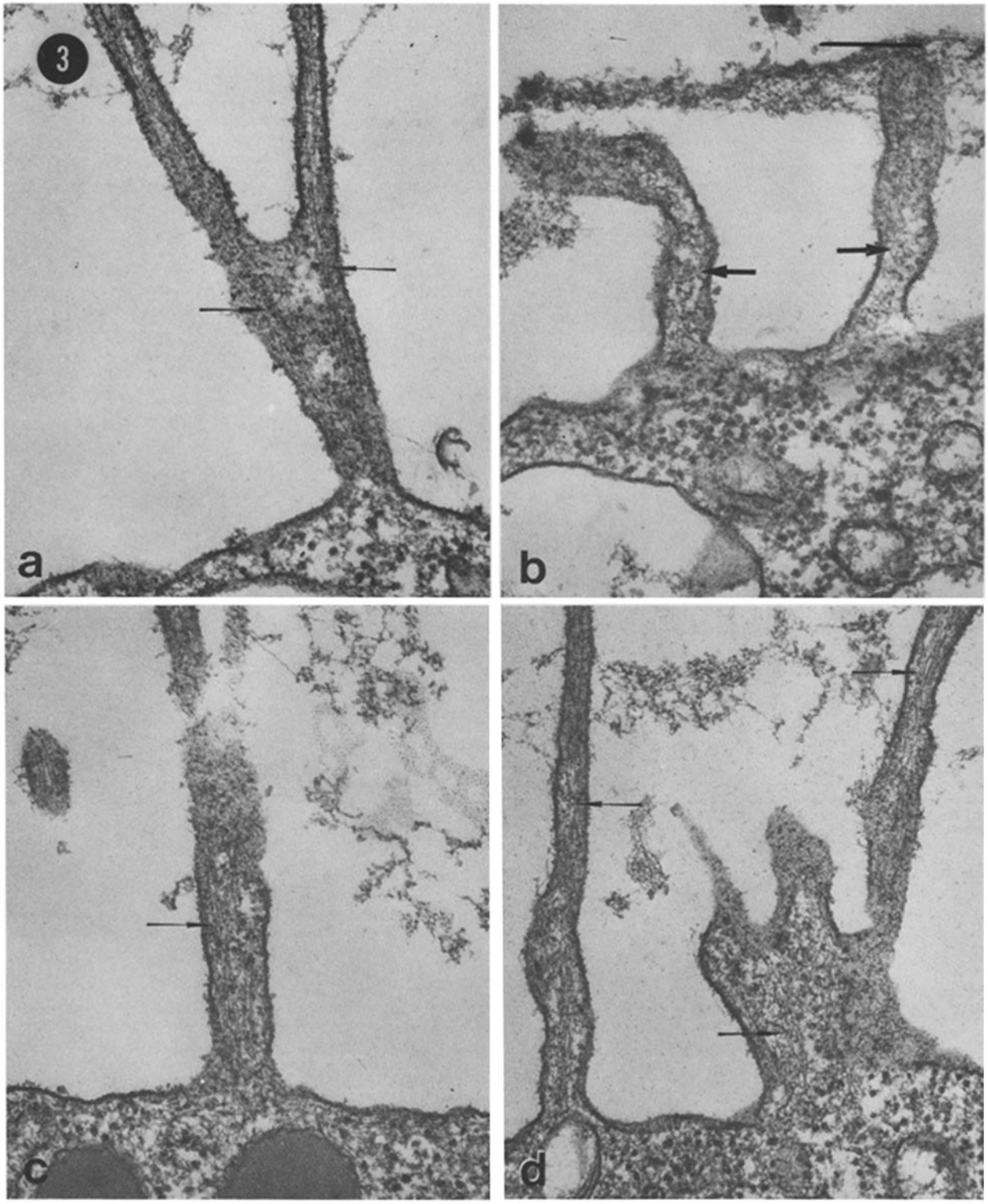

FIGURE 3 Transmission EM of *A. punctulata* eggs activated with A23187 in normal and Na^+-free ASW. (*a*) ASW. Arrows indicate filament bundles. (*b*) Choline-SW. The microvilli are irregularly shaped and bent. Arrows indicate filament network within the microvilli. (*c*) Aliquot of eggs from *b*, fixed 15 min after the addition of 40 mM NaCl. The microvilli are long and straight and contain bundles of actin filaments (arrow). (*d*) Aliquot of eggs from *b*, fixed 15 min after the addition of 10 mM NH_4Cl. The microvilli are indistinguishable from those shown in *a* or *c* and contain bundled actin filaments (arrows). Bar, 0.3 μm. × 60,500.

a Ca^{++} requirement for the formation of actin filaments in the isolated egg cortex may be an artifact induced by the isolation conditions. Alternatively, lysis of the egg may cause the release of an intracellular store of Ca^{++} which rapidly interacts with actin in the cortex before being bound by the EGTA in the isolation medium. As in the intact egg, this exposure to Ca^{++} could alter the actin so that it becomes capable of forming bundles of filaments at elevated pH.

Although we are unable to explain the apparent difference in the control of actin filament bundle formation in vivo and

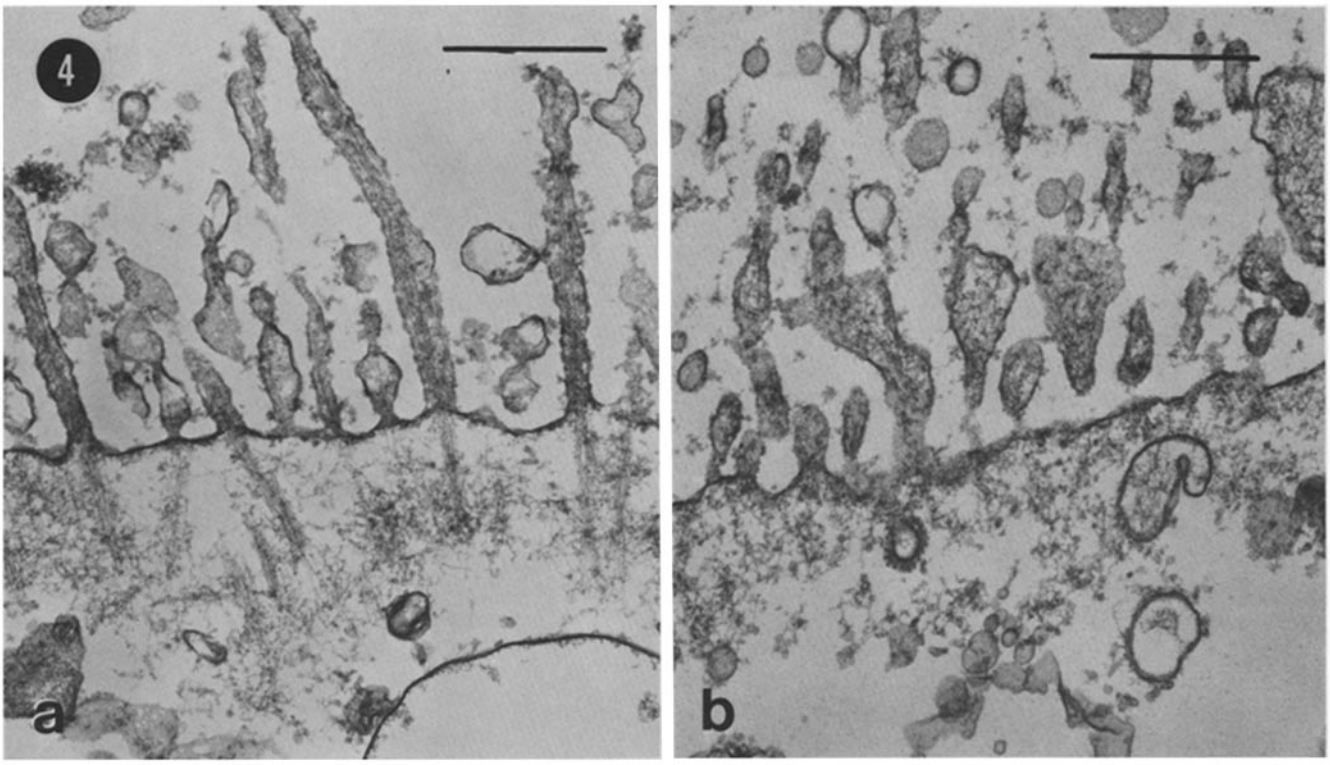

FIGURE 4 Cortices isolated from *S. purpuratus* eggs activated with A23187. (*a*) ASW. Cortex isolated 40 min after activation. Note the long, straight bundles of actin filaments within the microvilli. Bar, 0.5 μm. × 44,000. (*b*) Choline-SW. Cortex isolated 30 min after activation. The microvilli are irregular in shape and contain a dense network of actin filaments. Bar, 0.5 μm. × 46,400.

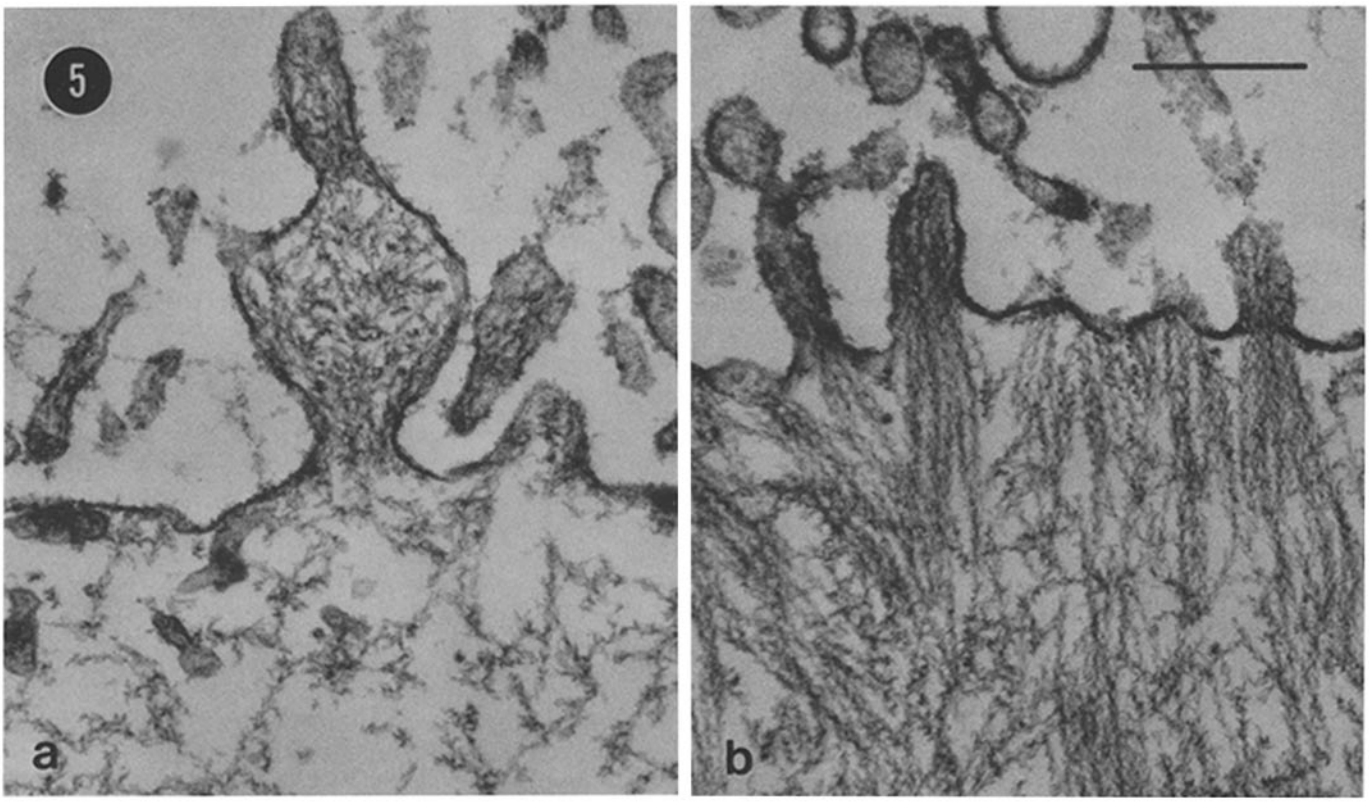

FIGURE 5 Myosin-S-1-decorated cortices isolated from *L. pictus* eggs at pH 6.5. Eggs were activated with A23187 in choline-SW. (*a*) Cortex isolated before addition of NaCl. Network of filaments binds S-1 to form arrowhead complex. (*b*) Cortex isolated from same preparation of eggs after addition of 40 mM NaCl. Microvillar core filaments show uniform basally oriented polarity typical of fertilized eggs. Bar, 0.3 μm. *a* and *b*, × 72,000.

in vitro, our results, as well as those of Carron and Longo (6), argue that microvillar elongation in the sea urchin egg is a two-step process involving both Ca^{++} and pH. The data presented here suggest that the increase in intracellular free Ca^{++} concentration induced by ionophore treatment causes the elongation of microvilli containing a network of actin filaments, while the subsequent increase in cytoplasmic pH elicited by the addition of NaCl or NH_4Cl transforms this filament network into bundles, with the concomitant conversion of the flaccid microvilli to their normal erect morphology. Cytoplasmic alkalinization alone, in the absence of an increase in free Ca^{++} concentration, is not sufficient to induce microvillar elongation or the formation of actin filament bundles in unfertilized eggs.

We propose that the same sequence of events observed under experimental conditions in Na^+-free SW occurs during normal fertilization in Na^+-containing media (Fig. 7). However, since cytoplasmic alkalinization rapidly follows the increase in intracellular free Ca^{++} concentration, these two events become superimposed. This interpretation is supported by the recent observations of Chandler and Heuser (13) on the initial stages of microvillar formation in *S. purpuratus* eggs. Using the techniques of fast-freezing, freeze-fracture, and deep-etching, they have studied the changes in the morphology of microvilli during the first 5 min after fertilization. In the first minute after fertilization, irregularly-shaped microvilli form which are similar to the flaccid microvilli we observe on eggs activated in choline-SW. Within 3–5 min after fertilization these microvilli assume their normal uniform shape. It is particularly interesting that the formation of irregular microvilli occurs during the period of transient Ca^{++} increase, while the transformation of irregular to straight microvilli takes place during the period of cytoplasmic alkalinization.

Tilney and Jaffe (38) have also proposed a two-step process of actin bundle formation to explain the elongation of sea urchin egg microvilli at fertilization. They observe that large numbers of actin filaments form within the cortex during the

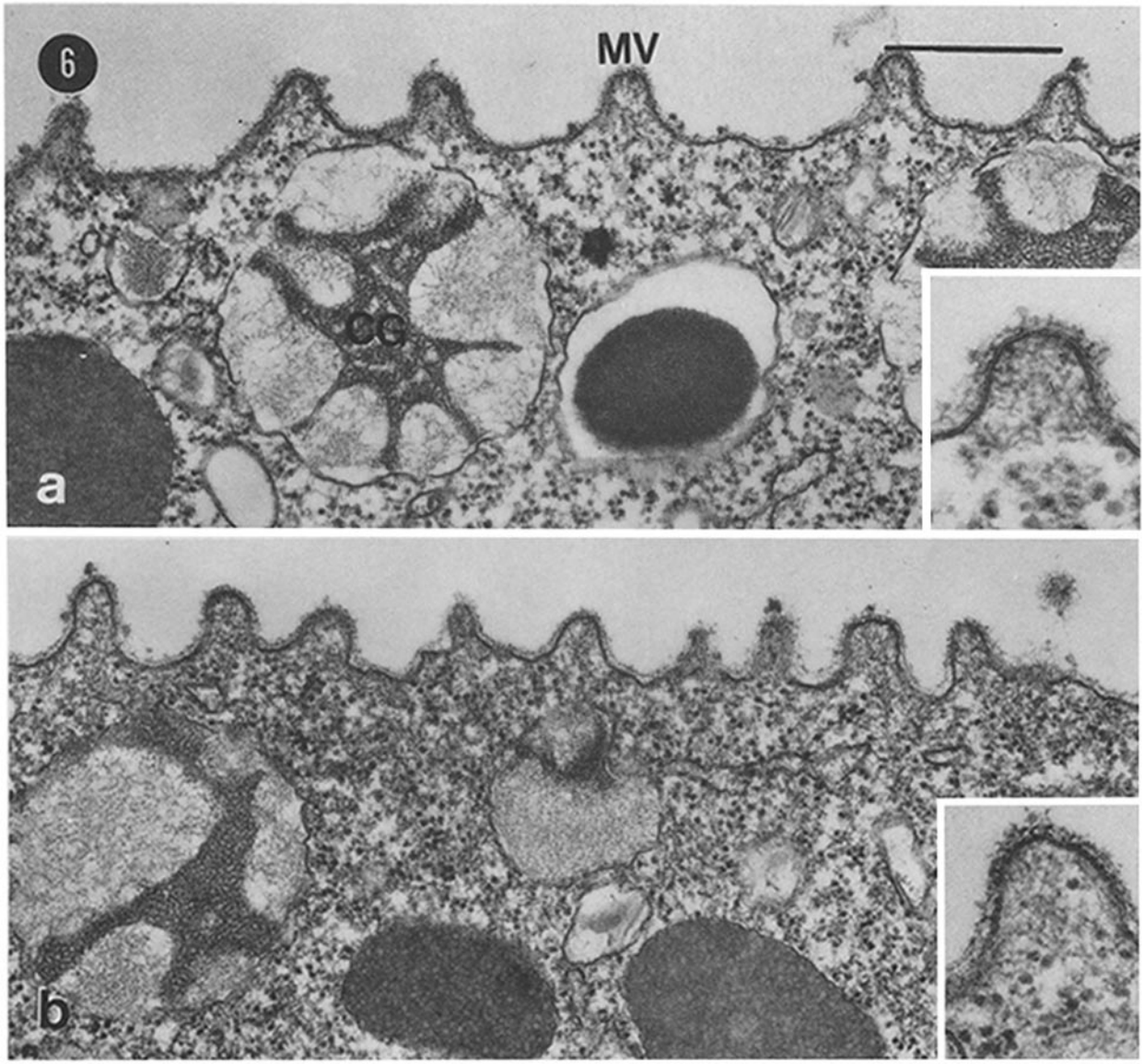

FIGURE 6 Unfertilized *Arbacia* eggs. (*a*) Untreated. Note the short microvilli (*MV*). CG-cortical granule. Bar, 0.5 μm. × 52,000. *Inset*: higher magnification of microvillus. × 130,000. (*b*) Aliquot of eggs shown in *a* after 30-min incubation in 10 mM NH_4Cl. The microvilli have not elongated. Bar, 0.5 μm. × 52,000. *Inset*: higher magnification view of microvillus showing absence of actin filament bundles. × 106,000.

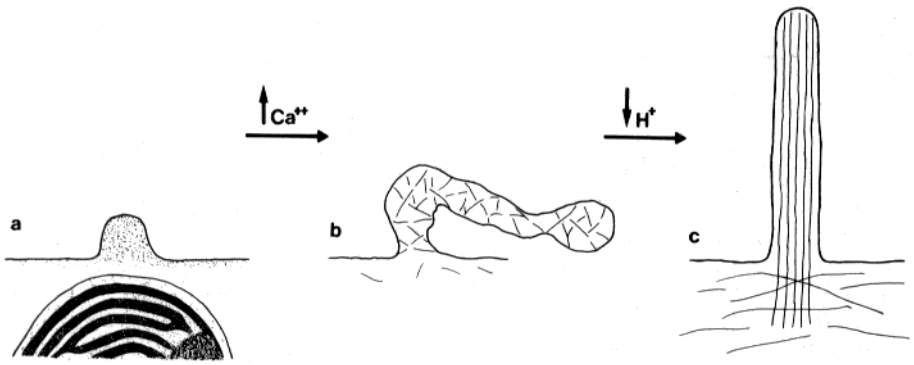

FIGURE 7 Proposed sequence of changes in actin organization associated with normal microvillar elongation. Actin is associated with the unfertilized egg cortex in an as yet uncharacterized storage state (*a*). At fertilization the transient increase in intracellular free Ca^{++} concentration induces the elongation of the microvilli and the transformation of cortical actin into a network within the microvilli (*b*). The subsequent increase in cytoplasmic pH causes a reorganization of the actin network into bundles of microvillar core filaments (*c*).

first 2 min after fertilization, but that bundles of microvillar associated actin filaments do not develop until ~5 min postinsemination. They propose that the actin filaments, once formed, are zippered together by cross-linking proteins to form bundles, and that the bundling process itself is responsible for the extension of the microvilli. This hypothesis is inconsistent with our data, which clearly demonstrate that microvillar elongation can occur in the absence of actin filament bundle formation. However, both reports agree that, unlike the formation of the acrosomal process (39, 40), filaments first form and subsequently become laterally associated into bundles.

A 58,000-dalton protein, named fascin, has been shown to cause the formation of actin filament bundles in extracts of sea urchin eggs (4) and in sea urchin coelomocytes (26). The recent demonstration by Otto et al. (27) that this protein is a component of the elongated microvilli of fertilized sea urchin eggs, but not of the unfertilized egg cortex, suggests that fascin may also cross-link actin filaments in the microvillar core. These observations raise the possibility that pH may regulate actin filament bundle formation by influencing actin-fascin interaction.

Our results demonstrate that microvillar elongation can occur in the absence of actin filament bundle formation but that longitudinally aligned bundles of filaments are required to provide the normal shape and rigid support for the microvilli once they are formed. That this may be the general mechanism of microvillar formation is suggested by the work of Chambers and Grey (8) on the development of intestinal epithelial cells in the chick. Microvilli first form on these cells as irregular projections of the apical plasma membrane containing a network of microfilaments and are subsequently converted into typical microvilli of uniform diameter with core bundles of actin filaments.

Although we do not know the actual mechanism of force production for microvillar extension, numerous possibilities exist. For example, the formation of a membrane-associated actin filament network may generate the force for microvillar elongation by a mechanism similar to that proposed for the extension of phagocytic processes in macrophages (21). Alternatively, a separate mechanism, such as increased hydrostatic pressure within the egg, may act as the force generator, while the actin network determines the shape of the developing protrusion. A third possibility, but one for which there is currently very little experimental evidence, is that changes within the plasma membrane itself might provide either the force or directional information for microvillar growth. While we cannot rule out any of these alternatives at this time, our results demonstrate that the generation of force for microvillar extension is not coupled to the formation of anisotropic bundles of actin filaments and suggest that cytoskeletal anisotropy may not be required for the protrusive activity of cells in general.

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Note Added in Proof: While this article was in press, nearly identical results were reported by Carron and Longo (1982. *Dev. Biol.* 89:129–137).

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