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Actin Filament Translocations in Sea Urchin Eggs

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Rhodamine (rh) phalloidin was used as a probe for actin filaments in living eggs and was observed using confocal microscopy. Exogenous, rh phalloidin labeled actin filaments were injected into eggs and were observed to translocate in the cytoplasm. At fertilization, filaments accumulated at the fertilization cone. Rh phalloidin alone, injected at concentrations that did not interfere with fertilization or mitosis, labeled filaments in the interior of unfertilized eggs, some of which translocated. At fertilization, staining increased dramatically at the fertilization cone and to a lesser degree around the entire cortex. At about 9 min, many filaments appeared to detach and translocated away from the egg surface. This study shows that there are mechanisms that can translocate actin filaments within the egg cytoplasm (at approximately 0.2–0.3 μm/sec), and raises the possibility that at least some of the changes in actin filament organization after fertilization are due to translocation of filaments.

Key words: phalloidin, confocal microscopy

INTRODUCTION

At fertilization of sea urchin eggs, there are large changes in actin organization. In unfertilized eggs, much of the actin appears to be unpolymerized, with short filaments present in small microvilli [Henson and Begg, 1988; Bonder et al., 1989]. After fertilization, there is a large increase in actin filaments at the site of sperm entry (the "fertilization cone"). This has been seen by electron microscopy [Tilney and Jaffe, 1980], by rhodamine (rh) phalloidin staining in fixed eggs [Cline and Schatten, 1986] and in living eggs injected with rhodamine conjugated actin [Hamaguchi and Mabuchi, 1988]. Also after fertilization, microvilli become much longer around the entire cortex and are seen to contain actin filament bundles [Mazia et al., 1975; Eddy and Shapiro, 1976; Burgess and Schroeder, 1977]. Electron microscopy also shows the development of a meshwork of actin filaments underlying the plasma membrane as well as actin filament bundles extending into the cytoplasm from the cell surface [Harris, 1968]. Other ways in which changes in cortical actin have been detected include a transient increase in cortical fluorescence in eggs injected with fluorescently labeled actin [Wang and Taylor, 1979; Hamaguchi amd Mabuchi, 1988], an increase in rh phalloidin staining seen in isolated cortices prepared from fertilized eggs [Yonemura and Mabuchi, 1987], and an increase in polymerized actin in detergent extracted eggs [Spudich and Spudich, 1979].

Confocal microscopy offers new opportunities for investigating actin organization because the optical sectioning allows higher resolution imaging of actin filaments within the egg. The results presented here show that actin filaments can translocate in the cytoplasm, and suggest that some of the changes in actin organization at fertilization are due to actin filament translocation.

MATERIALS AND METHODS

Lytechinus pictus were obtained from Marinus, Inc. (Long Beach, CA), and were maintained in running sea water at the Marine Biological Laboratory (Woods Hole, MA). Gametes were obtained by electric shock.

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The eggs were quantitatively injected using mercury loaded pipettes (Hiramoto, 1962) using techniques and an injection chamber as described previously (Kiehart, 1982; Terasaki and Jaffe, 1993). All experiments were done at 18–20°C.

Lyophilized rabbit muscle actin was a generous gift from D.J. Fishkind. Procedures to make and label actin filaments were similar to those described in Cao and Wang [1990b]. An aliquot (1 mg) was resuspended in 1 ml of buffer G (0.05 mM $CaCl_2$, 2 mM Tris HCl, pH 8) with 0.2 mM ATP, 0.25 mM DTT, and 0.02% sodium azide. This was transferred to dialysis tubing and dialysed against 1 L of the same solution at 4°C. The dialysis buffer was changed daily for up to 1 week. To assemble the actin filaments, 18 μl actin solution was added to 7 μl water and 25 μl of 2× assembly buffer (assembly buffer is 75 mM KCl, 2 mM $MgCl_2$, 2 mM Tris acetate, pH 7) containing ATP and DTT, and was incubated at room temperature for 1 h. To label the filaments, 50 μl of stock rh phalloidin was dried to ~2 μl (see below), to which was added 50 μl of assembly buffer with ATP and DTT. This was added to the polymerized filaments and incubated for 45 min on ice. The amounts of actin and phalloidin in the labeling mixture were about 4.3 μM and 3.4 μM respectively; at these concentrations, phalloidin should be completely bound to actin, since the affinity constant for rh phalloidin is 17–40 nM [Huang et al., 1992; De La Cruz and Pollard, 1994]. The filaments were sonicated briefly to reduce the number of long actin filaments in the preparation; long filaments appear to become clumped at the injection site and do not disperse. For most experiments, the labeled filaments were diluted 1:10 with the injection buffer before they were injected at 5% of the egg volume. Total actin concentration in eggs has been estimated by DNase inhibition assays to be 1.3 mg/ml in *Tripneustes gratilla* [Otto et al., 1980] and 3 mg/ml in *Strongylocentrotus purpuratus* [Mabuchi and Spudich, 1980]. The volume of *L. pictus* eggs is about 600 pl; if *L. pictus* has a similar concentration of actin, the total amount of actin would be 860–2,000 pg. The amount of rh phalloidin stabilized filaments injected (0.5 pg) would therefore be 0.06%–0.025% of the total cell actin.

Rh phalloidin was obtained from Molecular Probes (Eugene, OR). It was stored as a stock solution of 300 U in 1.5 ml methanol (6.6 μM). For injection, 50 μl was dried down with nitrogen to ~2 μl. To this was added 10 μl of injection buffer (100 mM potassium glutamate, 10 mM Hepes, pH 7). A 5% injection was made, so that the final concentration of rh phalloidin in the egg was about 1.4 μM.

Nocodazole was obtained from Sigma (St. Louis, MO), was stored as a 1 mg/ml stock in DMSO and was used at a final concentration at 0.02 μg/ml in sea water, a concentration that blocks sperm aster formation in fertilized eggs.

Observations were made with a laser scanning confocal microscope (Model 600, BioRad Laboratories, Oxnard, CA) coupled with a Zeiss Axioskop. A Zeiss 40× neofluar N.A. 1.3 objective lens was used. Images were stored either on the hard disk or on a Panasonic TQ-3038F optical memory disk recorder (OMDR). For on line acquisitions, a ForA video timer (VTG-33) was used to record the time on the images. In some experiments, the confocal microscope was set on continuous scan and each frame was recorded manually on the OMDR.

The 568 line of the krypton/argon laser was used for fluorescence excitation. The laser was used at full power with a 3% neutral density filter. The light energy was typically 50 μW at the back aperture of the objective lens (measured with a laser power meter; 1815-C Optical Power Meter, Newport/Klinger, Irvine, CA). The transmission of 568 nm light through the combination of lens, oil, cover slip, and water was measured as ~82%. In sequences where the microscope was set on continuous scan, the average energy per second divided by the area of the scanned region was 7.4 $nW/\mu m^2$ (zoom 3 magnification). At this radiation level, translocations and cytoplasmic movements appeared to continue normally for at least 5 min. Typical settings on the scan head were aperture 5 and PMT gain 800–950. At the slow scan setting, the time interval per full frame was 3.3 sec, though sequences were often obtained with half height frames with half the time interval. The zoom was usually between 1.5 and 3.

For tracking movements (Fig. 3), images from the OMDR were captured with a Scion LG-3 video card (Frederick, MD) in a Macintosh computer with program NIH Image (from Wayne Rasband, Research Services Branch, NIH, Bethesda, MD).

RESULTS

Observations With Injected Actin Filaments

Purified rabbit muscle actin was polymerized in vitro, labeled with rh phalloidin and then injected into unfertilized sea urchin eggs. By about 10 min after injection, short filaments were scattered throughout the cytoplasm (Fig. 1). In time lapse sequences, some of the filaments were observed to translocate (Fig. 2A). Approximately 1–5% of the filaments translocated while the rest appeared to be coupled with the slow cytoplasmic movements of yolk and endoplasmic reticulum; as previously described, these movements are over short distances in random directions about a central position [Terasaki and Jaffe, 1991; Terasaki, 1994]. The translocating filaments often moved along curved paths and did not always move at a uniform velocity (Fig. 3). Quanti-

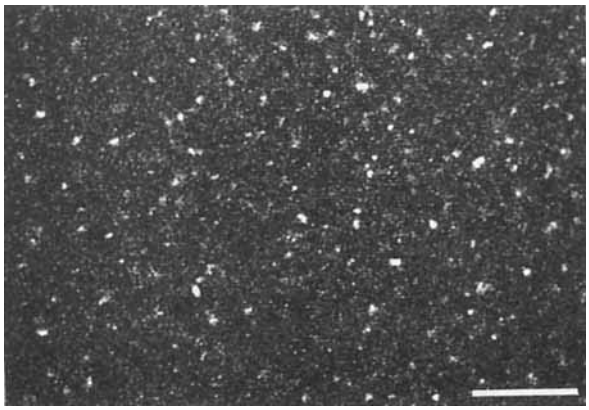

Fig. 1. Rh phalloidin stabilized actin filaments injected into a sea urchin egg. Actin filaments were polymerized from purified skeletal muscle actin and labeled with rh phalloidin. Within 10 min after injection into an unfertilized egg, the filaments spread throughout the interior of the egg, as seen in this confocal microscope image taken approximately 10 μm from the surface. Bar = 10 μm.

tative motion analysis would require taking into account the motion in three dimensions similar to the analysis done previously for pigment granule movements [Allen et al., 1992]. In preliminary measurements, made without making corrections for movements in three dimensions, the filaments moved at 0.33 ± 0.06 μm/sec (net rate; n = 14, SD, also see Fig. 2 legend). At fertilization, there is a contraction of the egg cortex [Hamaguchi and Hiramoto, 1980]; the translocations halted, then resumed at about 2.5 ± 0.8 min after the contraction began (n = 4 eggs).

The fertilization cone is a cone shaped projection from the egg surface that forms at the site of attachment of the fertilizing sperm [Fol, 1877]. Actin filaments appear in large numbers in the underlying cytoplasm, as shown by electron microscopy [Tilney and Jaffe, 1980], and by rh phalloidin staining of fixed eggs [Cline and Schatten, 1986]. In eggs injected with rh phalloidin stabilized actin filaments, there was a large increase in staining at the fertilization cone (Fig. 4; seen in 13 eggs) and sometimes at the cortex (seen in four eggs). If rh phalloidin remains bound to the actin filaments, the increased staining at the fertilization cone and cortex should be due to filaments in the interior that have moved and bound to these regions. Some filaments were seen to move and attach to the fertilization cone region, but there did not seem to be enough of them to account for all of the increase. The signal from the injected filaments at the fertilization cone began to become reduced at about 8.5 min and gradually became dimmer afterwards (n = 4 eggs). In two eggs, large groups of filaments also appeared to detach from the fertilization cone and move

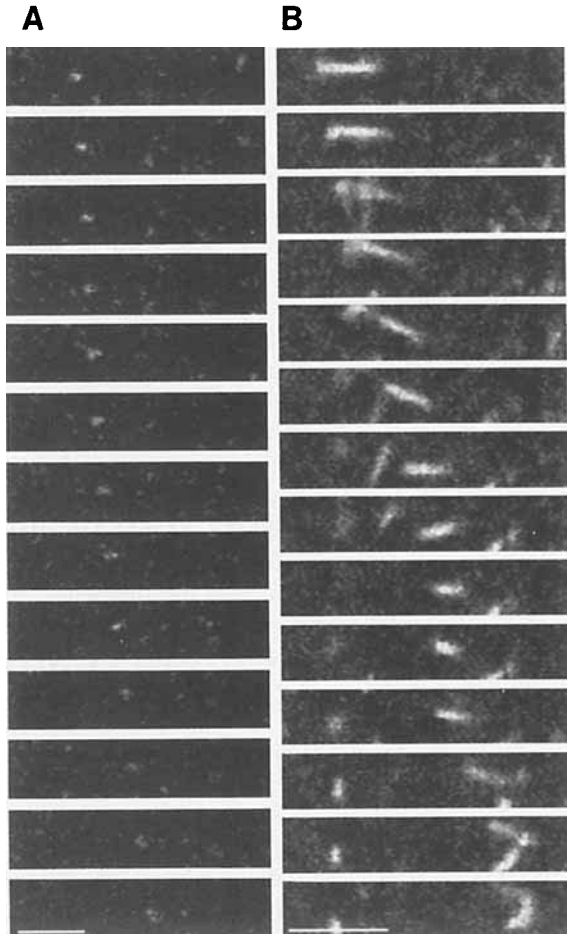

Fig. 2. Motility of injected filaments and rh phalloidin stained filaments. **A:** This is a time lapse view of a small region of cytoplasm of an egg injected with rh phalloidin labeled actin filaments showing translocation of an injected filament. The time interval between frames is 1.6 sec. To measure rates of translocation, three regions 66 × 44 μm in the same egg were imaged at 1.6 sec intervals for a total of 7 min 19 sec. Fourteen translocations were observed lasting for 6 to 22 sec (4 to 14 intervals). **B:** This is a similar view of an egg injected with rh phalloidin showing translocation of a filament. The time interval between frames is 7 sec. To measure rates of movement, a 34 × 80 μm region was imaged at 1.6 sec intervals for 3 min 7 sec. Ten clear translocations were observed, lasting from 8 to 17 sec (5 to 11 time intervals) with one lasting 31 sec. Bars = 5 μm.

inwards in a manner similar to that seen in eggs injected with rh phalloidin (see below).

Observations in rh Phalloidin Injected Eggs

Eggs were also injected with rh phalloidin at a final concentration about 1.5 μM. This concentration is low in comparison to the total actin concentration of 30–70 μM [Otto et al., 1980; Mabuchi and Spudich, 1980]. By 8 min, stained linear structures was clearly seen scattered throughout the cytoplasm (Fig. 5). The linear structures were not all of equal brightness, indicating that some or

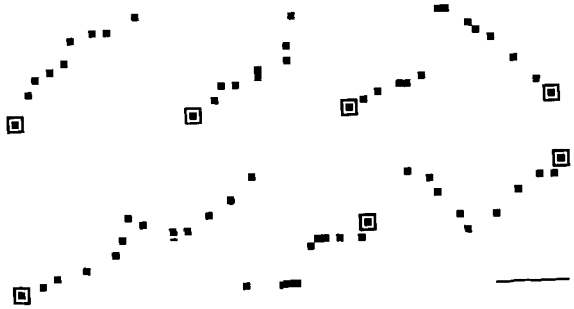

Fig. 3. Paths of translocating injected filaments. A region of an egg was imaged every 1.6 sec. An image processor was used to track the position of seven translocating filaments. Open squares are the first time point in the sequence. In one case (lower left corner), a filament was stationary for one time interval; this is indicated by an underlined square. The paths were often not straight or of uniform rate. Bar = 2 μm.

Fig. 4. Accumulation of injected filaments at the fertilization cone. An egg injected with rh phalloidin stabilized filaments was fertilized. Many injected filaments accumulated at the fertilization cone (approximately 2.5 min post-fertilization). Bar = 10 μm.

most may be bundles of actin filaments. These filamentous structures will be referred to as "filaments" with the understanding that they may be single actin filaments or bundles of actin filaments. In time lapse sequences, about 1–5% of the filaments translocated (Fig. 2B), while the rest appeared to be coupled to the slow cytoplasmic movements described previously [Terasaki and Jaffe, 1991; Terasaki, 1994]. The approximate rate of translocation (without taking into account motion in three dimensions) was 0.21 ± 0.06 μm/sec (n = 10, see Fig. 2 legend for details). The filaments are very probably not moving on microtubules because few if any microtubules are detected by immunofluorescence in unfertilized eggs [Harris et al., 1980] and the translocations still occurred in nocodazole (3/3 eggs). At fertilization, translocations were not seen during the first few minutes, then resumed at about 3.5 ± 0.4 min (n = 5 eggs) after the contraction associated with fertilization began.

There was a large increase in staining at the site of sperm entry, starting about 1 min after the fertilization

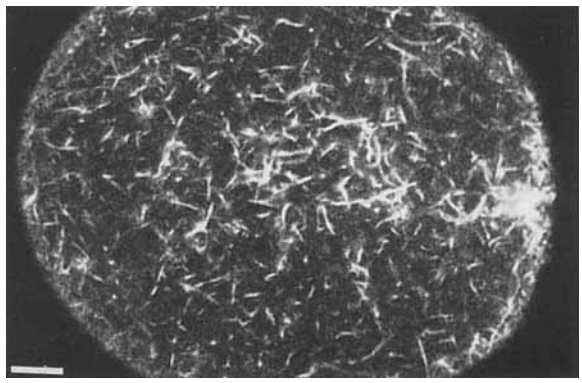

Fig. 5. Fluorescence image of a rh phalloidin injected egg. The egg was injected with a final concentration of 1.4 μM. Linear structures corresponding to actin filaments or actin filament bundles are seen distributed throughout the cytoplasm (this image was taken 8 min 30 sec after injection). There was a typically large amount of staining at the site of the needle penetration (see right side of egg) that gradually decreased with time. Staining at the site of injection was seen previously by Hamaguchi and Mabuchi [1982]. Bar = 10 μm.

envelope began to rise (Fig. 6; n = 24 eggs). A prominent array of filaments developed, extending about 10 μm at about 9 min after fertilization. These images usually seemed more distinct than the images in eggs injected with rh phalloidin stabilized actin filaments. Staining around the entire cortex increased with a similar time course, but extended a shorter distance, about 5 μm (Figs. 6, 7). Although a few translocations to the fertilization cone region and cortex were observed, there did not appear to be enough to account for all of the increase.

At about 9 min (9.2 ± 1.3 min, n = 6 eggs), filaments appeared to detach from both the fertilization cone region and the cortex and traveled inwards (Figs. 6E, 7B). The filaments from the fertilization cone region and the nearby cortex moved generally in the direction of the centrosome (Figs. 6, 7). Filaments from the other regions of the cortex traveled inwards also, often taking curved paths, but not towards the centrosome. The rates of movements of filaments from the cortex were 0.16 ± 0.08 μm/sec, n = 10 (measured in an egg from about 12 to 14.5 min after fertilization). In 6/8 eggs, a thinner region of staining at the cortex was left after the filaments began to move inwards (compare Figs. 6F and 6H, or 7C and 7D); in the other two cases, the cortical region remained thick even though many filaments appeared to have left it.

Nocodazole did not block the development of the staining at the fertilization cone region or cortex, and filaments came off from these areas and translocated within the cytoplasm without moving preferentially towards the sperm pronucleus/centrosome (3/3 eggs). In agreement with previous work [Hamaguchi and Mabu-

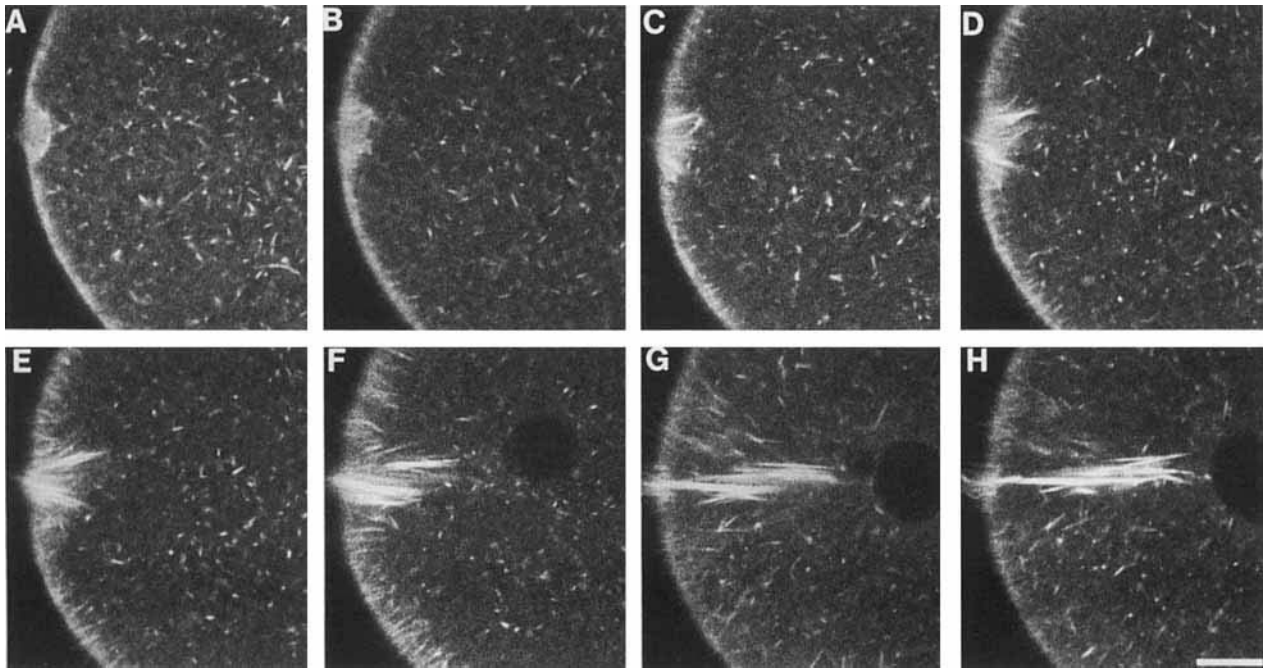

Fig. 6. Changes at the cortex during fertilization in a rh phalloidin injected egg. **A:** 1 min after the fertilization envelope began to rise. The fertilizing sperm was attached at the left, where there is increased staining. This is the future site of the fertilization cone. In subsequent images, the stained filaments became longer in this region and to a lesser degree around the entire cortex. **B:** 3 min 11 sec. **C:** 5 min 11 sec. **D:** 7 min 12 sec. **E:** 9 min 13 sec. This was approximately the time when the filaments at the fertilization cone and many filaments at the cortex appeared to come off of the cortex and migrate away from the egg surface. **F:** 13 min 20 sec. The egg pronucleus has come into the field of view and is visible as a dark circle about 12 μm in diameter. **G:** 20 min 46 sec. The sperm pronucleus (about 4 μm diameter) is barely detectable to the left of the female pronucleus. **H:** 23 min 17 sec. Pronuclear fusion has occurred. Bar = 10 μm.

chi, 1982], phalloidin injected eggs underwent mitosis normally (8/8 eggs).

DISCUSSION

Phalloidin, a bicyclic peptide toxin of *Amanita* mushrooms, binds to actin monomers within filaments, saturating at a 1:1 molar ratio, but does not bind to soluble, unpolymerized actin monomers [Wieland, 1977; Wieland and Faulstich, 1978; De La Cruz and Pollard, 1994]. An actin filament that is saturated with rh phalloidin can still serve as a substrate for myosin [Yanagida et al., 1984] and can still be severed by gelsolin or actophorin [Bearer, 1991; Maciver et al., 1991]. From studies in cell free systems, phalloidin at saturating concentrations decreases the off rate from the minus end of the actin filament, thereby lowering the critical concentration for actin filament assembly and stabilizing the filaments against depolymerization [Estes et al., 1981; Coluccio and Tilney, 1984; Sampath and Pollard, 1991; De La Cruz and Pollard, 1994].

Rh phalloidin has been used in two ways in living cells. In one way, Cao and Wang [1990b] made use of the stabilizing effects of rh phalloidin by labeling polymerized actin filaments in vitro with an approximately 1:1 ratio of rh phalloidin then injecting the filaments into cultured cells. The movements of the injected filaments will not necessarily be the same as native filaments, but they provide information about whether translocation systems exist and if so, about some of their characteristics. Using this technique, Cao and Wang [1990b] showed that filaments translocate to the mitotic cleavage furrow in normal rat kidney cells. This indicates the presence of mechanisms that can translocate actin filaments in these cells, and strongly suggests that the contractile ring is at least partly formed from the translocation of filaments.

In the second way of using rh phalloidin in living cells, rh phalloidin has been microinjected directly. High concentrations of rh phalloidin can interfere with normal cell functions [Wehland et al., 1977], but low concentrations of rh phalloidin to not block mitosis in sand dollar eggs (<4 μM; Hamaguchi and Mabuchi [1982]), fibroblasts (1–2 μM; Cao and Wang [1990a]) and *Tradescantia* stamen hair cells (0.27 μM; Cleary et al. [1992]). Rh phalloidin also does not interfere with cortical movements of *Aplysia* bag cell neuron growth cones (<~10 μM; Lin and Forscher [1995]). In the present

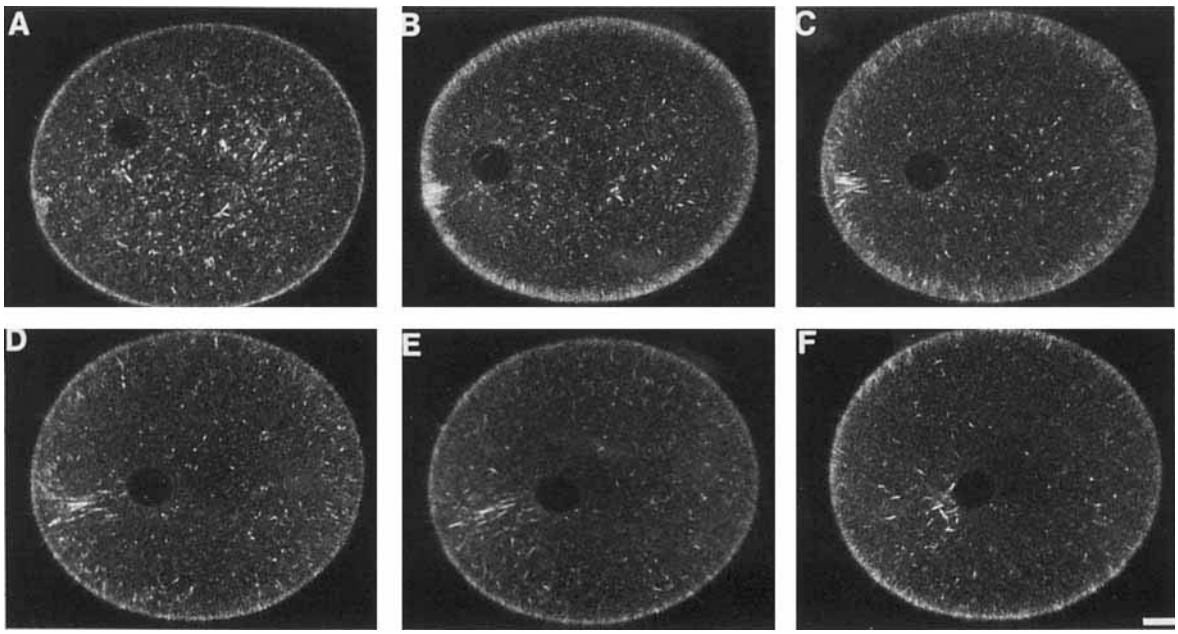

Fig. 7. Lower magnification view of a rh phalloidin injected egg during fertilization. Once the sperm centrosome has entered the egg cytoplasm, it nucleates a microtubule aster that serves to move the centrosome towards the center of the egg, and also serves to provide a track for the migration of the female pronucleus towards the centrosome; the male and female pronuclei subsequently fuse [see Wilson, 1928]. In this sequence, the female pronucleus and its movements were fortuitously in the same plane of focus as the fertilization cone. **A:** 1 min after the fertilization envelope became visible. There is increased staining at the sperm entry site on the left. The dark circle nearby is the female pronucleus. There is a filament within the nucleus. This was seen fairly often, as reported by Hamaguchi and Mabuchi [1982]. **B:** 10 min 50 sec. The female pronucleus has begun to move towards the sperm entry site. **C:** 13 min 10 sec. The filaments at the fertilization cone region have apparently detached; the beginning of the release was first detectable at about 11 min 4 sec. The width of staining around the entire cortex has increased significantly compared to the first panel. The female pronucleus has begun to migrate towards the center of the egg. **D:** 15 min 45 sec. Filaments from the fertilization cone and many filaments from the cortex near the fertilization cone moved towards the migrating pronuclei and aster complex. Filaments from other regions of the cortex moved inwards but generally not towards the aster. The staining around the entire cortex has decreased significantly compared to the previous panel. **E:** 18 min 19 sec. **F:** 26 min. Note that there are fewer filaments in the interior at this time than at the beginning of the sequence. Bar = 10 μm.

study, rh phalloidin was injected into the eggs at a final concentration in the egg of ~1.5 μM and had no obvious effects on fertilization or mitosis. Although low concentrations of rh phalloidin do not block cell functions, there are many uncertainties about how rh phalloidin interacts with actin in living cells and therefore many uncertainties about how to interpret the staining patterns. Some possible ways that rh phalloidin and actin might interact are: 1) rh phalloidin binds to stable native filaments and does not label or interfere with more dynamic actin filaments. In fibroblasts, rh phalloidin labeled the actin filaments in stress fibers but not in lamellipodia [Wehland and Weber, 1981; Wang, 1987], suggesting that rh phalloidin became bound to stable actin filaments in stress fibers and was not available to stain the more rapidly turning over actin filaments in lamellipodia; 2) rh phalloidin induces polymerization of some actin filaments and is tied up in stabilizing these filaments, allowing normal actin dynamics to occur; 3) actin filaments are labeled with sufficiently low substoichiometric (i.e. non-saturating) levels of rh phalloidin that normal actin polymerization/depolymerization occurs. The effects of substoichiometric levels of phalloidin have not been well studied, but in one report, a 0.1:1 molar ratio provides little protection against depolymerization [Dancker et al., 1975].

In the present study, both rh phalloidin stabilized actin filaments and rh phalloidin alone were injected into sea urchin eggs. Short, injected filaments were seen to translocate in unfertilized eggs. Rh phalloidin alone labeled filaments in the interior of unfertilized eggs. With the uncertainties about how rh phalloidin interacts with actin, it is not clear to what degree these filaments are native. One reason to think that they may be native comes from previous observations of eggs of the sea urchin *Arbacia punctulata*. In these eggs, some of the acidic organelles are pigmented. The pigmented organelles are distributed throughout the interior and undergo cytochalasin sensitive salatory movements [Belanger and Rustad, 1972; Allen et al., 1992]; the filaments in the rh phalloidin injected eggs may corre-

spond to the tracks for those movements. Whether or not the filaments in rh phalloidin injected eggs are native, some of them were observed to translocate. The translocations occurred over curved paths in three dimensions, and the precise rates were not determined, though approximate values for these filaments and for the injected filaments were 0.2–0.3 μm/sec.

The actin filament translocations seem likely to be due to movement by myosin molecules that are anchored in the cytoplasm. This would be similar to the widely used in vitro assay where actin filaments are translocated by myosin bound to a glass cover slip [Kron and Spudich, 1986]. Myosin is probably not bound to microtubules because the translocations occur in the presence of nocodazole. Myosin may be bound to another cytosolic structure, or it may be bound and immobilized in the endoplasmic reticulum. This membrane system could provide continuous pathways throughout the egg [Jaffe and Terasaki, 1993]. Movements involving ER and actin have been described in plant cells [Kachar and Reese, 1988; Allen and Brown, 1988; Knebel et al., 1990] (in these cases, ER membranes are thought to move along stationary actin filaments).

At fertilization, the injected filaments accumulated at the fertilization cone and in some instances around the rest of the cortex. The time course and appearance of the increases, particularly at the fertilization cone, is similar to that seen in eggs fixed then stained with rh phalloidin [Cline and Schatten, 1986]. This similarity suggests that the development of the fertilization cone is at least partly due to the translocation of pre-existing actin filaments from the interior. In rh phalloidin injected eggs, staining also increased at the fertilization cone, and invariably increased in the rest of the cortex. If rh phalloidin does become tightly bound to stable actin filaments in the unfertilized egg (see paragraph 3 of this section), the results also suggest that increases in cortical actin are at least partly due to translocation of pre-existing actin filaments from the interior.

There are two difficulties. In contrast to the later change at 9 min post-fertilization, translocations were not very clearly observed, so it was not possible to document that the increases at the fertilization cone and cortex were due to translocation. Second, all myosins characterized to date move actin filaments with the minus end leading, and should give rise to actin filaments with their minus ends at the plasma membrane, but in previous studies that used myosin subfragments to determine actin filament polarity, it was found that most actin filaments at the cortex and fertilization cone are oriented with their plus ends at the plasma membrane [Begg et al., 1978; Tilney and Jaffe, 1980]. If pre-existing filaments are translocated to the cortex, perhaps there is an uncharacterised motor protein that moves actin filaments with opposite polarity, or perhaps filaments that are translocated to the cortex comprise the smaller fraction of minus end oriented filaments. Another possibility is that there is significant turnover of rh phalloidin, even from the injected filaments, and that the increase in cortical rh phalloidin fluorescence is due to newly polymerized actin filaments labeled by a pool of free rh phalloidin rather than to translocation of filaments.

In the later change in cortical actin staining at about 9 min after fertilization, many actin filaments were observed to translocate from the cortex towards the egg interior. The translocations were most clearly seen in the rh phalloidin injected eggs. The direction of movement away from the cortex is consistent with movement of actin filaments with their + ends at the cortex by myosin. In sea urchin eggs (*Clypeaster japonicus*) injected with fluorescently labeled actin, a transient increase in cortical fluorescence after fertilization has been observed [Hamaguchi and Mabuchi, 1988]. The cortical fluorescence was maximum at 90 sec after insemination, and then returned to original levels by 5–6 min. The translocations from the cortex in rh phalloidin injected eggs may correspond with the decreasing phase of cortical fluorescence in eggs injected with fluorescently labeled actin. There is a discrepancy, however, of these studies in living cells with the results of rh phalloidin staining of fixed cells, because a decrease in cortical actin occurring after the initial increase at fertilization has not been reported. A study to optimize fixation conditions for preserving actin organization in fertilized sea urchin eggs could be useful in determining whether actin filament translocations from the cortex occur normally.

In conclusion, confocal microscopy is very well suited to observe rh phalloidin labeling in living sea urchin eggs. The observations show that there are mechanisms for translocating actin filaments in sea urchin eggs. The changes at fertilization suggest that the translocation mechanisms are regulated in order to move filaments to specific locations within the cell. This raises the possibility that translocation has a major role in actin organization and dynamics.

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