

POLARIZED BUNDLES OF ACTIN FILAMENTS WITHIN MICROVILLI OF FERTILIZED SEA URCHIN EGGS

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ABSTRACT

We report on the internal ultrastructure of long, finger-like microvilli which cover the surface of the fertilized sea urchin egg. Eggs were attached to polylysine-coated surfaces; their upper portions were sheared away with a stream of buffer which left behind only their plasma membranes and adjacent cytoplasmic structures. Scanning electron microscopy (EM) of such fragments revealed intact thin, protoplasmic projections radiating away from the body of the cortex. By transmission EM of cortices similarly prepared on grids, small bundles of microfilaments appear as cores within the thin cytoplasmic projections. These microfilaments are shown to be composed of actin by their ability to interact with muscle heavy meromyosin (HMM). HMM-decorated microfilaments possess repeating arrowheads which uniformly point toward the cell interior. Actin bundles in the microvilli of sea urchin eggs may mediate microvillus support and elongation.

KEY WORDS microfilaments · cortex · cytoskeleton · heavy meromyosin

Before cell division, the surface of the sea urchin egg is covered with protoplasmic projections. They have occasionally been observed in the past (2, 4, 5, 10, 11, 13) and recently confirmed with the aid of the scanning electron microscope (EM) (Schroeder, T. E., manuscript in preparation). Of the many thousands of finger-like microvilli per egg, about one-quarter rapidly grow to 5–10 μm in length at about 1 h after fertilization (Schroeder, T. E., manuscript in preparation). The finger-like microvilli are thought to be derived from shorter projections (0.5–1 μm in length) (22) which are formed from very short papillae present on the unfertilized egg (Schroeder, T. E., manuscript in preparation; see also references 8, 16, and 20). The growth mechanism of these protoplasmic projections is unknown, as is their function.

Many cell types possess finger-like projections termed microvilli. Brush-border microvilli facing

the intestinal lumen, for example, contain a core of unidirectionally polarized actin filaments that terminates in a meshwork of filaments called the terminal web (12, 17, 19). Bundles of actin filaments exhibiting the same polarity as intestinal microvillar core filaments have also been found within microspikes of platelets (18) and filopodia of echinoderm coelomocytes (7). The best evidence that protoplasmic projections of sea urchin eggs may resemble intestinal microvilli in substructure comes from an ultrastructural study by Harris (10). She found fine filaments within the projections which extended into the cortical region of the egg. There is as yet only suggestive evidence of a terminal web or tangential array of filaments in the cortex of the sea urchin egg (Burgess, D. R., manuscript in preparation; see also references 14 and 15). The cortical region of amphibian (9) and rat (1) oocytes has been shown to contain a significant amount of polymerized actin. In the amphibian oocyte, some of this actin appears to be orga-

nized into bundles within the short microvilli covering the egg surface (9).

In this communication, we report on an ultrastructural preparation of the egg cortex that retains the internal structure of the thin protoplasmic projections. Such cortices were prepared at stages well after fertilization when many of the finger-like projections have elongated. We positively identify bundles of actin filaments in these structures; we also show that these actin filaments are all polarized in the same way as core filaments in brush-border microvilli.

MATERIALS AND METHODS

Preparation of Eggs

Eggs of the purple sea urchin *Strongylocentrotus purpuratus* were inseminated, rinsed briefly in 1 M urea, and passed through 80- μ m nylon mesh to denude them of fertilization envelopes and hyaline layers. Eggs were raised as a 1% suspension in calcium-free seawater¹ (CaFSW) at 11°–13° C.

Cortices were prepared from eggs that had developed for 1 or 2 h. An aqueous solution of poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.; 1 mg/ml) was poured onto a substrate (cover slip or coated EM grid). The surface was rinsed in CaFSW and a volume of the egg suspension added to the surface. Adhering cells were rinsed gently with shearing solution (10 mM Tris, pH 8.0, 0.1 M NaCl, 5 mM EGTA, 5 mM MgCl₂). A stream of shearing solution from a squeeze bottle washed away the cells, except for the adhering cortex (3, 24).

Electron Microscopy

Whole eggs (fertilized and denuded) were prepared for scanning electron microscopy (EM) by fixation in 1% glutaraldehyde, 90% CaFSW, pH 7.2, and postfixation in 0.5% OsO₄ – 0.4 M Na acetate, pH 6.0. Samples were dehydrated, critical-point dried from Freon 13, coated with gold-palladium by evaporation, and examined in an ETEC Autoscan EM. Whole eggs were also prepared for transmission EM by fixation in 1% glutaraldehyde, 90% CaFSW, pH 8.2, and postfixation in 1% OsO₄ – 0.4 M Na acetate, pH 6.0.

Cortical fragments were prepared for scanning EM by fixation in 2% glutaraldehyde in shearing solution and postfixation in 1% OsO₄ – 0.4 M Na acetate, pH 6.0. These samples were critical-point dried, coated, and examined as described above. Cortices were also prepared

from eggs attached to grids coated with Formvar and carbon. Once sheared, cortices on grids were rinsed in shearing solution containing 0.5% Triton X-100; some of these were incubated for 5 min in 10 mg/ml heavy meromyosin (HMM) prepared from rabbit skeletal muscle. Grids were negatively stained with uranyl acetate. Transmission EM was done with a Philips EM300.

RESULTS

The surface of the fertilized premitotic sea urchin egg is densely covered with elongate finger-like projections when observed with the scanning EM (Fig. 1a). Most of these projections are longer than 1 μ m and many are 5–10 μ m in length. They are uniformly 0.10–0.15 μ m in diam.

The cellular surface of fertilized sea urchin eggs adheres tenaciously to polylysine-coated surfaces. Cells remain intact and stuck down during gentle rinsing in shearing solution. But when a jet of shearing solution is directed at them, the bulk of each cell is washed away. Only the adherent surface and immediately associated cytoplasm remains after shearing. Fig. 1b shows part of an isolated egg cortex observed with the scanning EM. Aside from the stuck-down plasma membrane, several large (1–2 μ m in diam) spheres are apparent within the perimeter of the cortex. These spheres are most likely echinochrome granules or residual cortical granules. Radiating from the periphery of the stuck-down cortices are many spine-like fibrils. The fibrils are thought to be surface microvilli which have adhered lengthwise to the substrate.

The substructure of protoplasmic projections from the egg surface becomes strikingly apparent when cortices are prepared on grids for transmission EM observation, especially after the grids are rinsed briefly in Triton X-100. The body of the cortex is composed of a dense anastomosing meshwork of fine filaments, as seen in Fig. 1c. Large numbers of projections, 1–10 μ m in length, are found at the periphery of these cortices (Fig. 1d). These projections are fairly straight, are uniformly 0.10–0.15 μ m in diam, and possess blunt tips. Many of these projections are still covered by loose sleeves of membrane.

A small bundle of 4.0- to 6.0-nm wide microfilaments courses the length of each projection (Fig. 2b and c). Core microfilament bundles usually remain intact, even in cases where the membrane appears to be completely solubilized. Each projection contains an average of 6.78 microfilaments (range = 5–10 microfilaments/microvillus; 23 microvilli counted; SD = 1.24). Evidence of a 12.0

¹ Calcium-free seawater (CaFSW), composed of NaCl (0.38 M), MgCl₂ (0.05 M), Na₂SO₄ (0.03 M), KCl (0.01 M), NaHCO₃ (0.002 M), Tris (hydroxymethyl) amino methane (Tris) (0.010 M), and ethylene glycol-bis(β -aminoethyl ether) *N,N,N',N'*-tetraacetate (EGTA) (0.0025 M), pH 8.0.

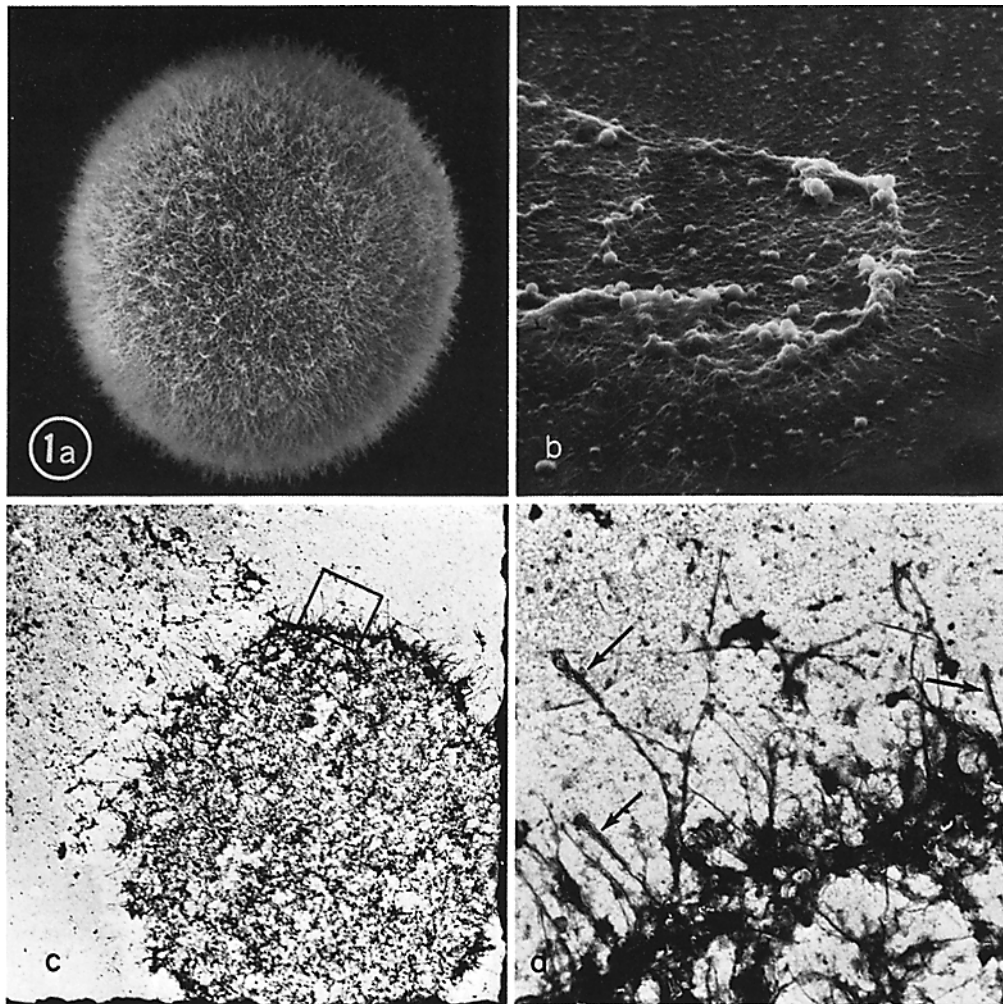


FIGURE 1 (a) Scanning electron micrograph of an egg that has developed for 2 h. Thousands of long, finger-like microvilli cover the egg surface. $\times 1,130$. (b) Inside of sea urchin egg cortex prepared 2 h after fertilization on a glass cover slip as described in Materials and Methods and viewed by scanning EM. Thin projections radiate away from the edge of the cortex. $\times 3,000$. (c) Transmission electron micrograph of a cortex prepared on a grid from an egg that had developed for 1 h. About one-third of the egg's surface is present. The remainder of the egg was washed away. The cortex appears as a coarse meshwork of fibers. The black border is formed by the bars of the 200-mesh grid. $\times 800$. (d) Higher magnification of the region outlined by the box in Fig. 1c. In this peripheral region of the cortex numerous short and long microvilli (arrows) radiate from the body of the cortex. $\times 6,800$.

± 0.5 nm axial periodicity was noted within some bundles where the filaments were closely apposed (Fig. 2c').

Microfilaments in finger-like projections can sometimes be detected in thin sections of whole eggs prepared in a way that is known to preserve microfilaments of the contractile ring (Fig. 2a). Where present, there are fewer than four or five microfilaments per projection; the bundles some-

times extend $1/2 \mu\text{m}$ into the cortex. Vesicles, sometimes in close proximity to the plasma membrane, are also observed in the cortical cytoplasm of sectioned eggs (Fig. 2a).

HMM treatment of cortices prepared on EM grids produces an easily recognizable arrowhead decoration of the microfilaments (Fig. 2d and e). All microfilaments of bundles in microvilli are decorated with "arrowheads" with an axial repeat

of 37 nm. Arrowheads on every filament within each projection are distinctly and uniformly polarized with the arrowheads pointing toward the body of the cortex and away from the microvillus tip. In many cases the sleeve of membrane around the filament bundle is completely solubilized by Triton X-100, yet a cap of material often remains at the distal tip of the bundle (Fig. 2*d*). It is not yet known whether this cap represents a particularly stable part of the membrane or a unique structure. The proximal ends of filament bundles are lost in a maze of decorated filaments in the body of the cortex.

DISCUSSION

The finger-like projections which cover the surface of the fertilized sea urchin egg are properly considered microvilli on the basis of their substructure as well as overall morphology. Like intestinal microvilli, egg microvilli have the same diameter along their entire length and have blunt, rounded tips. Each egg microvillus also contains a bundle of specifically polarized actin filaments which runs the length of the projection and inserts into the cortical cytoplasm, like brush-border microvilli (12, 17, 19). We found indications that egg microvilli possess some kind of terminal specialization from which core filaments originate; like the apical caps of brush-border microvilli, these specializations resist solubilization by Triton X-100, though further similarities have not been established.

Differences between egg microvilli and brush-border microvilli of intestinal epithelial cells include: (a) egg microvilli are less regular in length but sometimes considerably longer than brush-border microvilli; (b) there are fewer actin filaments per core bundle in egg microvilli (5-10 vs. 20-30); and (c) axial periodicity in egg microvilli filament bundles is 12.0 nm vs. 33.0 nm for intestinal microvilli filament bundles (17). It is interesting to note that actin filament bundles prepared from gelled extracts of sea urchin eggs have a distinct axial periodicity of 12.5 nm (6). Further significance of these differences, with regard to either physiological or mechanical properties of microvilli, is unknown at this time. It is apparent from the results presented here, however, that microvilli from differing cell types are highly conserved organelles as far as basic internal structure is concerned.

It is possible that egg microvillus actin performs several roles, e.g., (a) a cytoskeletal role in supporting microvilli as rigid structures (see refer-

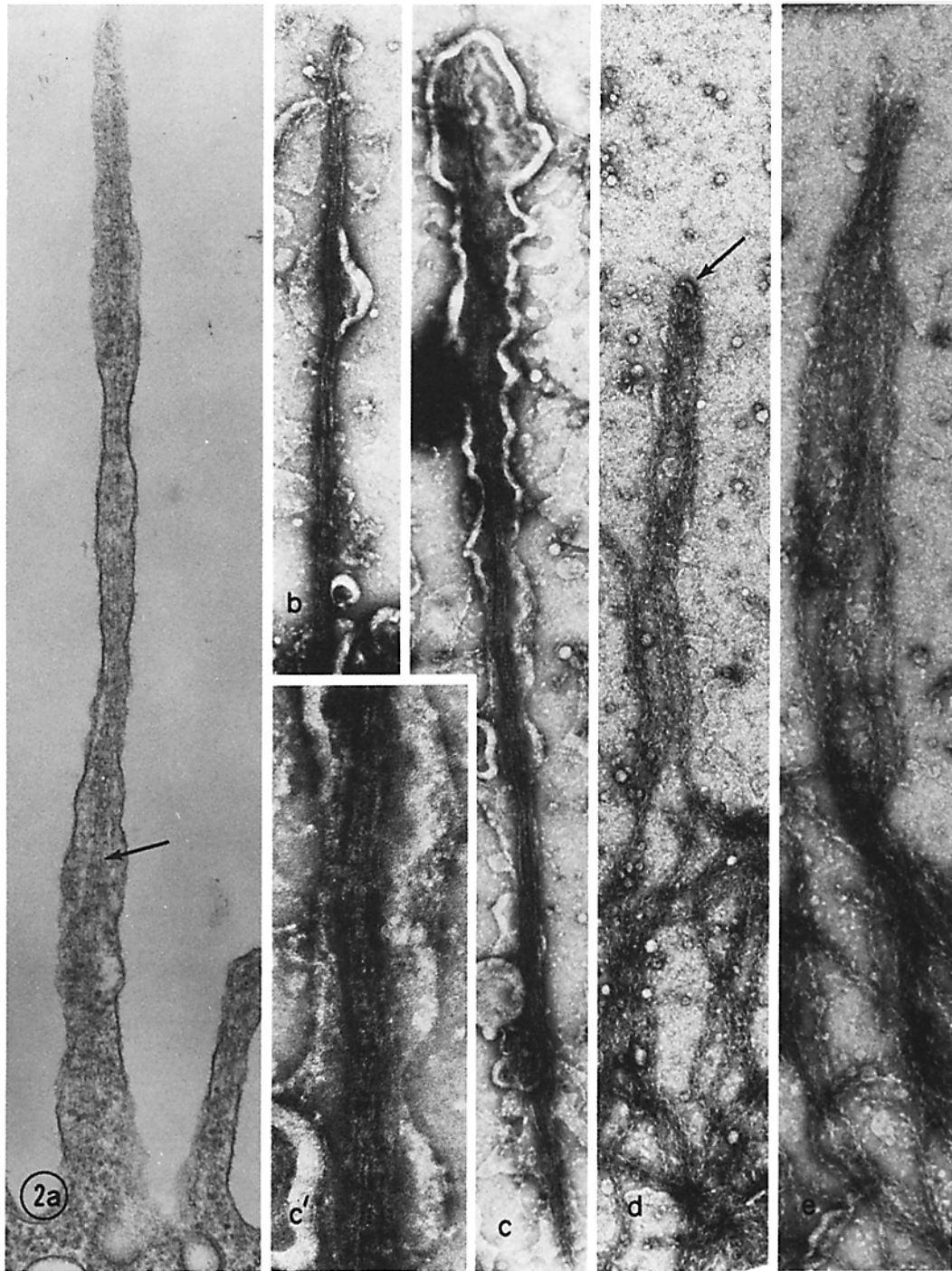
ences 17 and 23). If true, it suggests that the bundles are anchored somehow in the egg cytoplasm, perhaps by a cytoskeletal component in the cortex (Burgess, D. R., manuscript in preparation; see also references 14, 15, and 20); (b) A constructive role in the assembly of the contractile ring microfilaments used for cleavage (21). Bundles of actin filaments could be recruited, perhaps by myosin rods, to form the contractile ring; (c) A role in the elongation of the microvilli. The dynamics of microvillus elongation may be a function of actin polymerization, as has been postulated for elongation of acrosome filaments in some sperm (23). The actin bundles, being rigid, would be ideal structures for mediating microvillus outgrowth. Such an increase in microvillus length 1 h after fertilization (Schroeder, T. E., manuscript in preparation) also suggests that there need be a concomitant increase in the amount of plasma membrane. The addition of new membrane to the plasmalemma may be one way of controlling microvillus growth. These matters, and the relationship of microvillus actin to other pools of actin, remain to be explored. Additional comparative and experimental work is required in order to understand the significance of egg microvilli, their dynamic elongation, and the role of their actin substructure.

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FIGURE 2 Transmission electron micrographs of microvilli from eggs that had developed for 2 h. All except Fig. 2c'. $\times 50,000$. (a) Thin section showing four or five fine filaments (arrow) running the long axis of the microvillus. Vesicles are present in the cortical cytoplasm adjacent to the plasma membrane. (b) Bundle of filaments from a microvillus in which the surrounding membrane has been mostly solubilized by Triton X-100. Evidence of periodicity between closely associated filaments is present in the upper one-third of the bundle. Specimen not treated with HMM. (c) Long microvillus possessing a core filament bundle with the surrounding sleeve of membrane almost completely intact. Specimen not treated with HMM. A region showing periodic cross-banding is present in the middle of the bundle. (c') Higher magnification of the region of 12.0-nm periodic cross-banding in the core filament bundle shown in Fig. 2c. $\times 150,000$. (d) Microvillus core from an HMM-treated cortex. Distinct periodic (37 nm) arrowhead decoration shows uniform directionality of core filaments. All arrowheads point downward, away from the tip. A small bit of material is present at the tip (arrow) while the core filament bundle merges proximally (below) with a loose meshwork of decorated filaments. (e) Another HMM-treated cortex showing distinct periodic and directional decoration of microvillar core filaments.