MICROFILAMENTS IN THE POLAR LOBE CONSTRICTION OF FERTILIZED EGGS OF *ILYANASSA OBSOLETA*

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INTRODUCTION

Polar lobe formation is a phenomenon of precisely timed changes in cell shape which precede and accompany cleavage in the fertilized eggs of several mollusks and annelids (Figs. 1 and 2). The polar lobe contains no nucleus, but possesses considerable developmental information (4-6). This paper will focus on the mechanism by which these cytoplasmic protuberances form and will implicate structures in the cortical cytoplasm (9) in this phenomenon.

This process by which a spherical egg is deformed roughly to a dumbbell shape and then, by lobe resorption, to a sphere again is overtly similar to cytokinesis. It may be separated experimentally from cytokinesis, however, since it can occur independently of nuclear control (15, 29) and is not associated with asters or a spindle apparatus (14).

Polar lobes in the embryos of *Ilyanassa obsoleta* can be prevented from forming or be made to resorb by treatment with cytochalasin B[1] (7, 19), a drug which in many cases alters the ultrastructure of microfilaments (21–23, 27) but not microtubules. Most of the polar lobe formation process, however, can occur without inhibition in the presence of colchicine or vinblastine[1] (7, 19), drugs which disrupt microtubules, but not microfilaments (2, 3, 13, 26, 27). We therefore have proposed that polar lobe formation is dependent upon microfilament function[1] (7, 19). Similar hypotheses have been made for cytokinesis in those organisms where the plasma membrane is permeable to the drug (10, 21, 22). However, since cytochalasin B also directly or indirectly affects such processes as nucleoside and sugar transport across membranes (12, 17), phagocytosis (30), and hormone secretion (28), it was necessary to determine by electron microscopy the extent to which polar lobe constrictions normally were associated with microfilaments. The present study demonstrates by three different methods of fixation the presence of a ring of microfilaments in the cortical cytoplasm at the base of the third polar lobe constriction.

[1] Conrad, G. W., and D. C. Williams. 1973. Submitted for publication.

MATERIALS AND METHODS

Specimens of *I. obsoleta* Stimpson (*Nassarius obsoletus* Say) were collected off the coast of Mount Desert Island, Maine or were purchased from the Supply Department of the Marine Biological Laboratory, Woods Hole, Mass. Snails were maintained either in fresh running sea water or in sea water aquaria using Instant Ocean salts. Egg capsules were collected and handled according to Costello et al., (8), and fertilized eggs were reared in pasteurized, filtered sea water or in Millipore-filtered artificial sea water containing 0.1 g/liter penicillin and streptomycin.

Eggs were prepared for electron microscopy in three different ways. *Method 1:* Eggs were fixed at room temperature for 45 min in solutions of 2% glutaraldehyde, pH 7.1, followed by 15 min at 2°–4°C. Glutaraldehyde solution (1,080 mosm) contained 0.081 M sodium phosphate, 0.185 M NaCl, 0.145 M KCl, and 10^{-5} M $MgCl_2$. Eggs were then rinsed in cold buffer solution (1,398 mosm) containing 0.15 M sodium phosphate, 0.342 M NaCl, 0.268 M KCl, and 10^{-5} M $MgCl_2$. They next were treated with cold solutions of 2% OsO_4 in the same buffer (18), rinsed in 0.1 M maleate buffer (pH 5.4), stained for 45 min in 0.5% aqueous uranyl acetate in maleate buffer, dehydrated in acetone, and flat embedded in Spurr's ERL resin formulation B (Hard) (24). Sections were stained with lead citrate (20) or 1% aqueous uranyl acetate followed by lead citrate. *Method 2:* Eggs were fixed by the method of Kalt (11): 3% glutaraldehyde, 2% formalin, 1% acrolein, and 2.5% dimethyl sulfoxide in 0.05 M sodium cacodylate buffer, pH 7.2, at room temperature for 12 h. Eggs were then rinsed in 0.1 M sodium cacodylate buffer at the

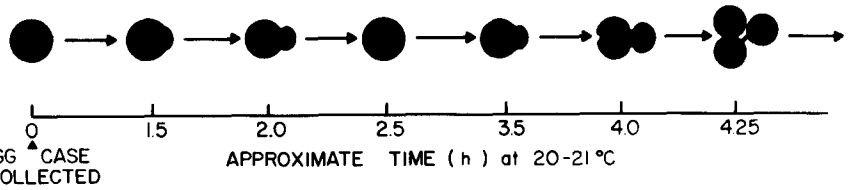


FIGURE 1 Changes in shape of the fertilized *Ilyanassa* egg before and during first cleavage. Soon after the fertilized egg is laid the first polar lobe, inconspicuous and transient (not shown), is formed directly opposite the location of the first polar body, which appears at the same time. About 30 min after the withdrawal of the first lobe, a second, prominent polar lobe appears and persists for about 1 h. The pronuclei fuse at the time of resorption of the second lobe. The third lobe forms about 40 min after the disappearance of the second lobe, at the time of spindle formation. This lobe is slightly larger than the second one. When the third polar lobe neck begins to tighten rapidly, the first cleavage furrow appears at the pole opposite the lobe. As the cleavage furrow deepens, the connection of the polar lobe with the blastomeres becomes very tenuous (trefoil, last stage; Fig. 2). As cleavage is completed, the neck of the polar lobe rapidly increases in diameter and as a result the lobe is resorbed by the cell defined as the CD blastomere. A fourth polar lobe (not shown) forms on the CD blastomere at the time of second cleavage.

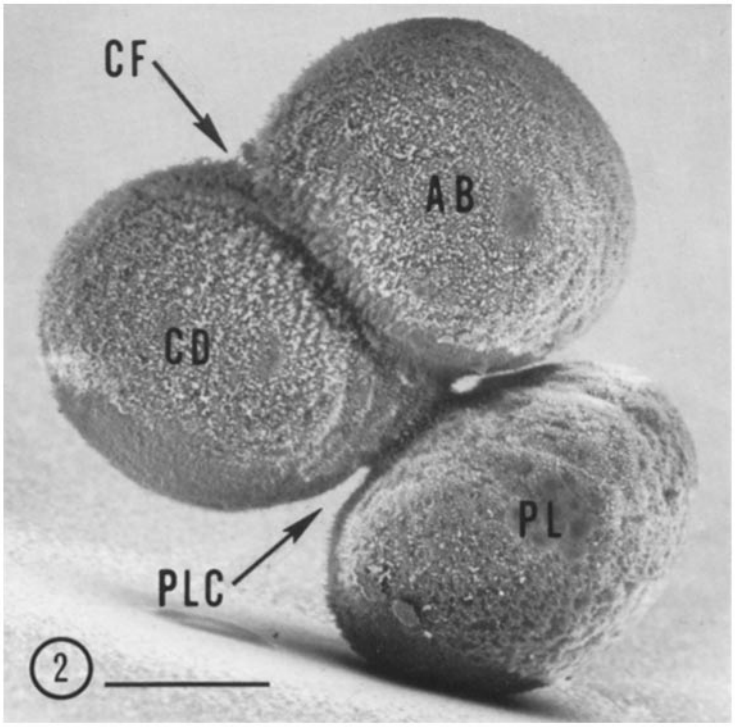


FIGURE 2 Scanning electron micrograph of an egg approximately in trefoil stage. The surface is covered with microvilli. The *AB* and *CD* blastomeres are indicated, as is the third polar lobe (*PL*), the first cleavage furrow (*CF*), and the polar lobe constriction (*PLC*). × 430. Bar, 50 μm.

same pH, postfixed at 4°C for 8–12 h with 1% OsO_4 in the 0.1 M cacodylate solution, rinsed with water, stained for 6 h with 1% aqueous uranyl acetate, dehydrated in ethanol, and embedded as in method 1. Sections were poststained with 1% aqueous uranyl acetate and Reynolds' lead citrate (20). *Method 3:* Eggs were fixed in 1% OsO_4 in sea water for 2 h and stained and embedded as in method 2. Blocks were sectioned with diamond knives on a Reichert ultramicrotome III (C. Reichert, sold by American Optical Corp., Buffalo, N. Y.) or a Porter-Blum MT1 (Ivan Sorvall, Inc., Newtown,

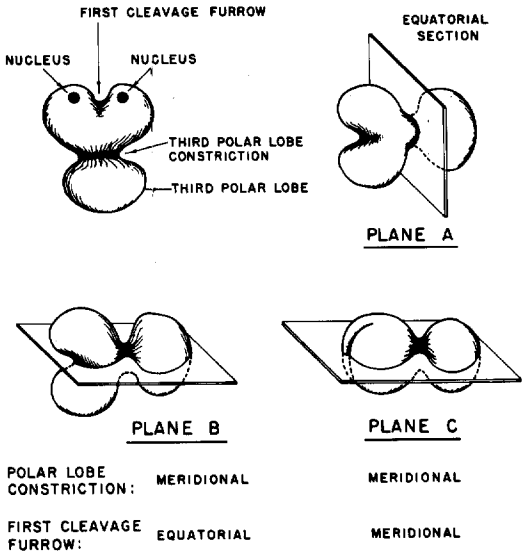


FIGURE 3 Diagram illustrating the orientation of planes of sectioning through a cleaving *Ilyanassa* egg.

Conn.). Sections were examined at 100 kV in a RCA EMU-4, at 60 kV in a Philips 201, or at 50 kV on a Hitachi HU-11A electron microscope.

For scanning electron microscopy, eggs were fixed in 6% glutaraldehyde in sea water, dehydrated in ethanol, transferred to amyl acetate, and critical-point dried. They were coated with a thin layer of AuPd alloy and examined with an ETEC Autoscan electron microscope.

Unless otherwise stated, eggs were fixed by methods 1–3 at a stage shortly after the first cleavage furrow had begun and after the third polar lobe constriction had begun to constrict tightly (Fig. 1, penultimate stage). Sections were made either in the plane of the polar lobe constriction (equatorial section) or perpendicular to this plane (meridional section) (21) (Fig. 3). Meridional sections of the polar lobe constriction cut the cleavage furrow region either in an equatorial plane or in a meridional plane. Sections also were made in planes parameridional (21) to the polar lobe constriction and cleavage furrow.

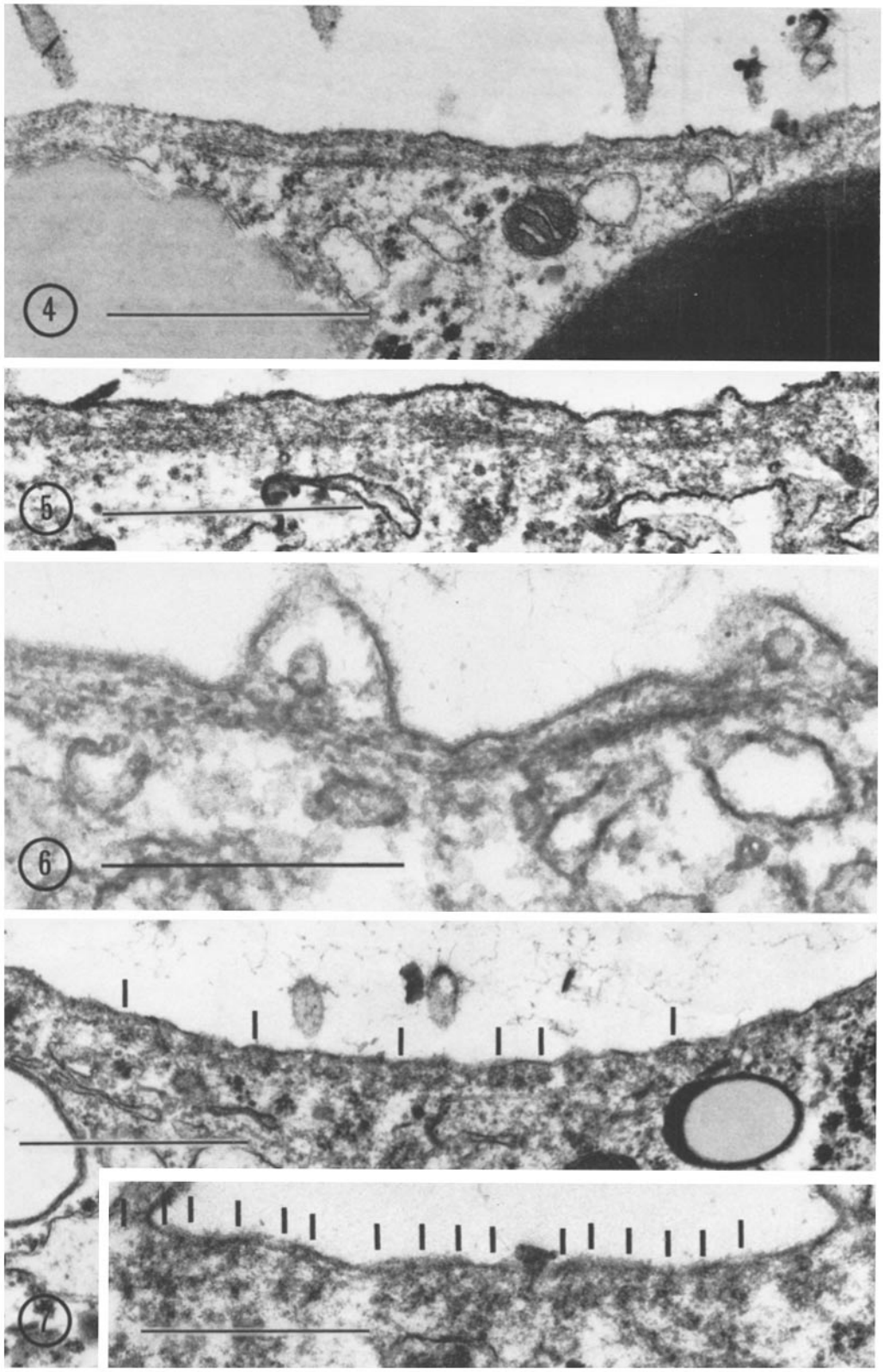
RESULTS AND DISCUSSION

A ring of microfilaments is associated with the base of the third polar lobe constriction in the fertilized egg of *Ilyanassa*. These microfilaments are arranged equatorially in the cytoplasm immediately apposed to the plasma membrane. In equatorial sections, the filaments can be seen running parallel to the plane of sectioning (Figs. 4–6), whereas in meridional sections the filaments are seen at right angles to the plane of sectioning and therefore appear as dense masses of material (Fig. 7). The diameters of these microfilaments (fixation method 1) as seen in equatorial sections are in the range of 30–50 Å.

Many investigators have demonstrated a ring of microfilaments at the base of the cleavage furrow of several types of animal cells (1, 21, 22, 25). As a control, we examined the base of the first cleavage furrow of the *Ilyanassa* egg to determine the extent to which microfilaments were present. As in the polar lobe constriction, equatorial sections of the cleavage furrow contained microfilaments running parallel to the plane of sectioning (Fig. 8), whereas in meridional sections they were seen in cross section as dense masses. The diameters of these cleavage furrow microfilaments (fixation method 1) as seen in equatorial sections were in the range of 30–50 Å. Although a quantitative study was not made, examination of meridional sections suggested a greater density of microfilaments in the cleavage furrow than in the polar lobe constriction. Also,

FIGURES 4–6 Equatorial sections through the neck of a third polar lobe (plane A, Fig. 3). Microfilaments associated with amorphous material are seen in the plane of the section, running in the cortical cytoplasm beneath the plasma membrane. Sections are from three separate eggs. Fig. 4: method 1; × 40,000. Fig. 5: method 1; × 80,000. Fig. 6: method 3; × 92,400. Bar, 1 μm in Fig. 4; 0.5 μm in Figs. 5 and 6.

FIGURE 7 Meridional section through the neck of a third polar lobe (plane C, Fig. 3). Microfilaments are arranged perpendicular to the plane of sectioning and are gathered in dense masses, some of which are indicated by bars, in the cortical cytoplasm apposed to the plasma membrane. *Inset:* A section serial to that in Fig. 7. Dense masses, somewhat separated from the plasma membrane but still within the cytoplasmic cortex, are seen to consist of amorphous material together with small dots or holes interpreted to be cross sections of microfilaments. Dense masses occur as indicated by bars. Method 1 × 35,100. *Inset:* × 70,000. Bar, 1 μm in Fig. 7; 0.5 μm in *inset*.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whereas the microfilaments appeared as a continuous mass in meridional sections of the cleavage furrow, similar sections through the polar lobe constriction showed microfilaments arranged in discontinuous masses (Fig. 7).

At the trefoil stage, a later time in development when the polar lobe neck has constricted maximally, filaments were still demonstrated easily in the cleavage furrow (Fig. 9) but were difficult to find in the polar lobe neck (Fig. 10). This suggests that the ring of microfilaments in the polar lobe constriction may be present only when the neck is actually decreasing in diameter. Regions of plasma membrane other than the cleavage furrow and polar lobe constriction were not associated with microfilaments, except

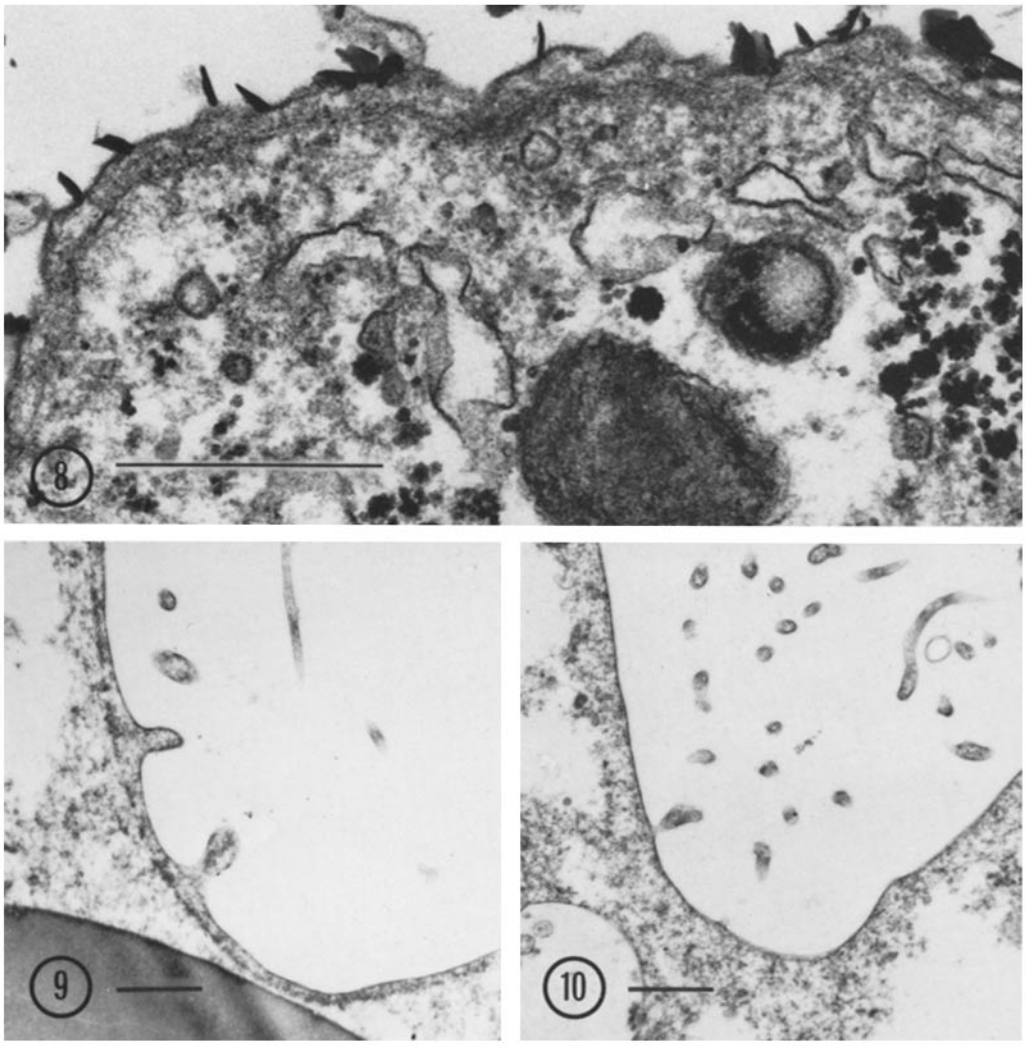

FIGURE 8 Equatorial section through the first cleavage furrow (plane B, Fig. 3). Microfilaments associated with amorphous material are seen in the plane of the section, running in the cortical cytoplasm beneath the plasma membrane. Method 1. × 70,000. Bar, 0.5 μm.

FIGURES 9 and 10 Meridional sections from a single trefoil stage embryo prepared by method 2 (plane C, Fig. 3). Fig. 9: first cleavage furrow; × 10,920. Fig. 10: third polar lobe constriction; × 10,920. Note the microfilaments beneath the plasma membrane in the cleavage furrow but their absence in the polar lobe constriction at this stage of development. Bar, 1 μm.

within the numerous microvilli (Fig. 2) which cover the surface of the embryo.

The results of this study of polar lobe formation suggest that, as in the case of cytokinesis, the furrowing of the cell surface occurs concomitantly with the presence of a ring of microfilaments apposed to the plasma membrane. Whether these filaments are actin-like, as are those in cleavage furrows (16), remains to be demonstrated. The extent to which the ultrastructure of the *Ilyanassa* egg is modified by treatment with cytochalasin B and colchicine will be described in detail elsewhere.[1] We suggest that polar lobe formation in the *Ilyanassa* egg offers an excellent model system for study of the regulation of cell shape.

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