

MECHANISM OF CELL SHAPE CHANGES IN FERTILIZED EGGS OF *Ilyanassa obsoleta*

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The fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta*, undergo a series of shape changes, called polar lobe formation, before and during early cleavage. Polar lobe constrictions resemble cleavage furrows morphologically, but they persist for a much longer time than cleavage furrows and do not cleave the cell completely into two parts, for each constriction eventually relaxes. Previous work has shown that a band of microfilaments lies at the base of each polar lobe constriction, analogous to the microfilamentous band at the base of cleavage furrows. Both types of bands are disassembled by treatment of the eggs with cytochalasin B, but not by treatment with colchicine. We have attempted to determine the extent to which the microfilaments in the polar lobe constriction are actin-like and the extent to which normal polar lobe formation and cytokinesis are dependent upon the presence of exogenous Ca^{+2} .

Myosin was purified from rabbit skeletal muscle, treated with trypsin, and subjected to ultracentrifugation. Heavy meromyosin (HMM) was purified from the supernatant solution. *Ilyanassa* eggs at various stages of polar lobe formation and cytokinesis were extracted with solutions of glycerol and then rinsed in buffer. Such extracted eggs were incubated with HMM in the presence and absence of ATP or sodium pyrophosphate, then fixed in glutaraldehyde and osmium tetroxide, embedded in SPURR resin, sectioned, stained with uranyl acetate and lead citrate, and examined with the electron microscope. In the absence of HMM, undecorated microfilaments are seen in the cortical cytoplasm at the base of the polar lobe constriction. In the presence of HMM, these microfilaments become decorated with arrow-heads. Negatively-stained samples of the HMM solution itself also were examined; no contaminant thin filaments (microfilaments) of muscle actin were detected. Thus, there appear to be actin-like microfilaments at the base of polar lobe constrictions in *Ilyanassa* eggs.

To detect actin-like protein in these eggs in another manner and to quantitate it during development we have used polyacrylamid slab gel electrophoresis. At specific stages of polar lobe formation and cytokinesis, samples consisting of 1000-2000 eggs were collected, gently disrupted with a detergent solution to release (but not lyse) yolk granules, and centrifuged. The supernatant solutions were separated from the pellet (yolk granules). Both fractions then were mixed with 9 volumes of acetone to precipitate proteins. Both fractions were centrifuged and separated into acetone supernatant solutions and pellets (precipitated proteins). All solutions and pellets were air-dried. These acetone powders were resolubilized with solutions of SDS and urea. Aliquots were analyzed for total protein (Lowry) and subjected to SDS-polyacrylamid slab gel electrophoresis, followed by staining with Coomassie blue. In the acetone precipitate from the fraction which does not contain the yolk granules, a major band is seen which co-migrates with standard chicken skeletal muscle actin. No actin-like band is seen in the yolk granule fraction or in either acetone supernatant fraction, although other proteins are present in such fractions. These results therefore support the conclusion from the electron microscopic studies that an actin-like protein is present within *Ilyanassa* eggs. Densitometric scanning of photographic transparencies of the stained gels to quantitate the amount of actin-like protein during development is in progress.

We have assayed the role of Ca^{+2} in polar lobe formation in two ways. First, we compared the response of eggs to microiontophoretic injection of Ca^{+2} to that in response to injected Sr^{+2} . Second, we determined the ability of the eggs to undergo polar lobe formation and resorption, as well as cytokinesis, in Ca^{+2} "free" solutions.

Previous work (Conrad, G. W. and S. E. Davis, *Develop. Biol.* (in press, December 1977)) has shown that *Ilyanassa* eggs will form a cytoplasmic protuberance that greatly resembles a normal polar lobe within 15-30 sec following injection with Ca^{+2} by microiontophoresis. Sr^{+2} can substitute directly for Ca^{+2} in

activating muscle contraction (accumulation by sarcoplasmic reticulum, activation of myofibrillar ATPase, and binding to troponin-tropomyosin) and generates muscle tension which is at least 90% of that generated by Ca^{+2} . Sr^{+2} also can substitute for Ca^{+2} in causing *Ilyanassa* eggs to form a lobe-like protuberance upon microiontophoretic injection. The timing and morphology of the response to injected Sr^{+2} are the same as to injected Ca^{+2} .

When *Ilyanassa* eggs are transferred from normal sea water, rinsed in Ca^{+2} , Mg^{+2} free artificial sea water (CMF-sea water) and then dispensed to solutions of normal sea water, CMF-sea water, or CMF-sea water + 10 mM EDTA, a majority of the eggs still form and resorb third polar lobes and undergo first cytokinesis in synchrony with control eggs left unrinsed in normal sea water. In CMF-sea water, approximately 70% of the rinsed eggs underwent normal development, whereas in CMF-sea water + 10 mM EDTA, approximately 65% developed normally (results of eight experiments). To determine whether the eggs in these CMF solutions were forming polar lobes and cleaving in the complete absence of both Ca^{+2} and Mg^{+2} , we analyzed the solutions by atomic absorption spectroscopy before and after they were used on the eggs. CMF-sea water + EDTA contained 3 μM Ca^{+2} ; after incubation with the eggs, such solutions contained 4-5 μM Ca^{+2} . CMF-sea water + EDTA contained 1 μM Mg^{+2} ; after incubation with the eggs, such solutions contained 3-4 μM Mg^{+2} . If Ca^{+2} (and Mg^{+2}) are required for normal polar lobe formation and cytokinesis, the results obtained here suggest that they are derived from intracellular sources or are required in only very low exogenous concentrations.

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TENSIONS EXERTED BY CLEAVAGE FURROWS OF *Echinorachnius parma* EGGS TREATED WITH CYTOCHALASIN B

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Tensions exerted by the cleavage furrow of sand dollar *Echinorachnius parma* eggs can be measured with calibrated glass needles (Rappaport, Science 156:1241, 1967). The force exerted by the normal furrow of the first cleavage is about 1.5×10^{-3} dyne. Because the furrow contracts isometrically, the force measured by this method represents the maximum amount available in the division mechanism rather than the amount that is actually required for division. In this investigation we attempted to ascertain the least force that could accomplish division by measuring forces exerted by furrows that had been debilitated by chemical treatment. After each measurement the needles were removed from the cell to determine whether or not it could complete division.

The antibiotic Cytochalasin B reverses already functioning cleavage furrows at the same time that it causes the disappearance of the microfilamentous subsurface ring which is located at the base of animal cleavage furrows (Schroeder, Biol. Bull. 137:413, 1969). Concentrations of about 0.10 $\mu\text{g}/\text{ml}$ slow division without blocking it and alter the response of the surface to manipulation. The surface became more sticky and fragile. At these concentrations the surface of all Cytochalasin B-treated eggs was altered, but eggs from different females could differ in their sensitivity. The glass bottom of the operation chamber was covered with a thin film of Lubriseal, which discouraged cell adhesion and subsequent rupture. There was also some tendency for cytoplasm to leak from the egg at the point of entrance of the needle, but leakage did not usually interfere with cleavage, and it eventually stopped. Old eggs appeared more susceptible to treatment than freshly ovulated eggs.