

REGULATION OF CELLULAR SHAPE CHANGE
IN FERTILIZED EGGS OF ILYANASSA OBSOLETA

Gary W. Conrad and Roberto R. Patron
Division of Biology, Ackert Hall, Kansas State University,
Manhattan, KS 66506

Fertilized eggs of the marine mudsnail, Ilyanassa obsoleta, change shape several times before and during first and second cleavage in a manner that mimics cytoplasmic division (cytokinesis). This pattern of shape change is termed polar lobe formation and resorption. During the summer of 1986, we continued our studies of the role of Ca^{2+} and of membrane-associated contractile elements in these cellular shape changes.

Ca^{2+} : Earlier work had demonstrated a lack of effect of several organic Ca^{2+} uptake antagonists, as well as La^{3+} , and Ni^{2+} on the cell shape changes. This study has now been completed with observations on the effects of Co^{2+} and Cd^{2+} . Fertilized eggs incubated in Millipore-filtered ($0.45 \mu\text{m}$) natural sea water or in nominally Ca^{2+} -free sea water (Ca^{2+} salts and Ca^{2+} -chelators omitted; Mg^{2+} salts present) undergo normal schedules of polar lobe formation and cytokinesis even in the presence of 1-10 mM CoCl_2 . The two cell shape changes still occur normally in natural sea water containing 1 mM CdCl_2 , but fail to occur at 10 mM. Similarly, although the normal shape changes can occur in Ca^{2+} -free sea water containing 0.1 mM CdCl_2 , both shape changes are prevented by 1 and 10 mM CdCl_2 . These data, like those collected previously, do not clearly support a requirement for exogenous Ca^{2+} for these shape changes.

Among calmodulin antagonists, our previous work has shown polar lobe formation and cytokinesis to be insensitive to 5-100 μM trifluoperazine but sensitive to 25-100 μM chlorpromazine in the presence or absence of exogenous Ca^{2+} . Calmidazolium is reported to be 500x more potent than trifluoperazine in inhibiting calmodulin-dependent phosphodiesterase and it was used here to resolve the apparent conflict between the effects of trifluoperazine and chlorpromazine. Normal polar lobe formation and cytokinesis occur even in sea water containing up to 10 μM calmidazolium or in Ca^{2+} -free sea water containing up to 1 μM calmidazolium. These data therefore are consistent with the relative insensitivity of the shape changes to trifluoperazine and suggest that the shape changes may not involve calmodulin.

Membranes: In order for microfilament-dependent processes to alter cellular shape, it is presumed that some microfilament-associated proteins must interact with the plasma membrane. During the summer, 1985, we began studying this putative membrane role in fertilized Ilyanassa eggs. We examined the effects of a brief treatment with digitonin and showed that it prevented polar lobe formation without inhibiting cleavage. Because some other detergents are thought to be even more specific than digitonin in binding cholesterol (Kinne-Saffran, E. and R. Kinne. 1979. J. Memb. Biol. 49:235-251), during the summer, 1986, we performed similar studies with them. When cells are treated for 2-6 min with 5 μM tomatine, they do not form polar lobes, but cleave on schedule in a manner generating equal cleavage, an effect very similar to that of digitonin. When cells are treated instead with a structural analog of tomatine that does not bind cholesterol, tomatidine, at even 20 μM for 8-10 min periods, normal polar lobe formation and cytokinesis still occur. Filipin (5-7 $\mu\text{g/ml}$) treatment for 2-5 min prevented polar lobe formation, but also frequently prevented cytokinesis in the same cells. These

data suggest that the polar lobe-forming mechanism is differentially sensitive to digitonin and tomatin, but no more sensitive to filipin than the apparatus accomplishing cytokinesis. Neither process is sensitive to tomatidine. We speculate that disturbance of normal cholesterol arrangement in the plasma membrane may account for the inhibition of both microfilament-dependent processes studied here. Supported by NIH grant HD07193.