

FREE CALCIUM ION CONCENTRATION RISES AT THE ONSET OF CELLULAR CONSTRICTIONS IN  
ILYANASSA OBSOLETA

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Previous work has shown that microfilaments are organized in the polar lobe constriction of fertilized eggs of Ilyanassa obsoleta Stimpson (Nassarius obsoletus Say) (Conrad et al., Dev. Biol. 36, 363-378, 1974). Morphologically similar contractile shape changes have been induced experimentally by  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ , cAMP and calcium ionophores (Conrad et al., Dev. Biol. 61, 184-201, 1977; Dev. Biol. 74, 152-172, 1980). Thus, a role was indicated for the involvement of calcium ions in cellular constrictions. The experiments reported here focused on examining the free calcium ion concentration in the cytosol at various sites in embryos forming third polar lobes and undergoing first cleavage. Because we knew where the cleavage furrow would form in these embryos, we could examine free calcium ion concentration at the site of cellular shape change before, during, and after it occurred. Calcium-selective microelectrodes (Tsien and Rink, J. Neurosci. Methods 4, 73-86, 1981) with tips of approximately 1.5  $\mu\text{m}$  outer diameter were used to probe both furrowing and non-furrowing regions of cytoplasm. Embryos were examined more easily in artificial seawater solutions containing low free calcium ion concentration and high osmolarity (raised 100 mM with sucrose). Development in such solutions was normal.

Our data indicate that the onset of the first cleavage furrow and that of the third polar lobe constriction are associated with a rise in free calcium ion concentration restricted to the site of the initiating constriction. When extracellular free calcium ion concentration was greater than 111  $\mu\text{M}$  the intracellular free calcium transients were large (66 to 240  $\mu\text{M}$ ) but short-lived (approximately two minutes' duration), even though polar lobe formation and cleavage last considerably longer. In contrast, when extracellular free calcium ion concentration was between 5 and 55  $\mu\text{M}$ , the constriction-associated free calcium ion transients appeared as a series of barely detectable waves which lasted for the first 8 to 10 minutes of cleavage. Non-furrowing regions of cytosol maintained resting levels of free calcium ion concentration at all times. These data suggest that calcium ion transients may be associated with constrictions in these embryos. Further work is required to determine if the free calcium transients are associated with the constrictions throughout their progression through the cytoplasm. (Research supported by NIH Grant HD 07193).