THE EFFECTS OF HEAVY METALS ON CYTOSKELETAL COMPONENTS INVOLVED IN CELL SHAPE CHANGES DURING FIRST CLEAVAGE IN ILYANASSA OBSOLETA

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During first cleavage in the marine mollusk, Ilyanassa obsoleta, two contractile rings are formed at right angles to each other in the fertilized egg. The cleavage furrow (CF) forms at the animal pole of the egg, equidistant between the two mitotic asters, and constricts across and around the animal hemisphere of the egg, in a plane perpendicular to the mitotic spindle axis, to form an intercellular bridge containing microfilaments (MFs), composed of F-actin, encircling midbody microtubules (MTs). The CF remains permanently constricted and eventually cleaves the two daughter cells apart from one another. The polar lobe constriction (PLC) forms in the vegetal hemisphere, beneath the mitotic apparatus, and constricts around the vegetal hemisphere of the egg, in a plane parallel to the mitotic spindle axis, to form a very tight polar lobe neck eventually containing no MFs and encircling no MTs. Normally, the PLC is a transient constriction and eventually relaxes, such that the polar lobe vegetal cytoplasm merges with one of the two daughter cells that formed from the animal hemisphere. In the presence of 5-7 X 10^{-11} M Ag^+, generated from diluting saturated solutions of $AgNO_3$ in sea water, the usually transient PLC becomes very elongated, encircles many MTs, becomes stably constricted, and often results in permanent separation of the polar lobe from the animal hemisphere cell to which it was attached (Conrad, AH, et al. 1994. Cell Motil. Cytoskel.27:117-132).

In order to demonstrate that it is the Ag^+ ions that cause the PLC stabilization, and not some other ion present as a contaminant in the $AgNO_3$ sea water solutions, silver wires (99.99% Ag^0) were used as (+) and (-) electrodes in separate electrode baths connected by an agarose bridge prepared in Millipore-filtered (0.45 μm) sea water (MFSW) to generate Ag^+ in the anode MFSW bath by electrolysis. After electrolysis, each electrode solution was adjusted to pH 7.7, as necessary, then diluted with more MFSW, and used to incubate fertilized eggs. The anode solution, containing free Ag^+, generated elongated stabilized PLCs, whereas the cathode solution did not alter PLC dynamics, compared to control eggs. In addition, gold, present as 3.5 μm $AuCl_3$ in MFSW, caused elongation and stabilization of the PLC during first cleavage, in a manner similar to that of Ag^+, whereas gadolinium, present at 1 mM $GdCl_3$ in MFSW, had no effect on cleavage or polar lobe formation.

In the previous silver study, it was postulated (a) that the presence of MTs in the PLC at maximum constriction allows the PLC contractile ring to retain MFs, as does the CF at maximum constriction when it encircles midbody MTs, and (b) that the retained MFs stabilize the constriction of the normally transient PLC. To test this hypothesis, Ilyanassa obsoleta fertilized eggs were allowed to develop in 5.2 X 10^{-11} M Ag^+-MFSW to the time of maximum CF/PLC constriction, then extracted, fixed, and triple-stained with antibodies to α-tubulin (localized with TRITC-labeled secondary antibody) to visualize MTs, FITC-labeled phalloidin to visualize F-actin, and Hoechst 33258 to visualize DNA. Control eggs showed F-actin in the CF and across the top of the polar lobe and into the PLC during early cleavage and PL neck constriction. However, at the time of maximum constriction, F-actin was still visible in the intercellular bridge of the CF around the midbody MTs, but no F-actin nor MTs were visible in the PLC. In

contrast, in the presence of Ag^+, both F-actin and MTs were visible in the CF and in the elongated PLC at the time of maximum constriction. We conclude that Ag^+-treated cells not only show increased numbers of MTs, but, judging from the immunofluorescence data, may show increased arrays of MFs as well. The latter conclusion must be confirmed by electron microscopy. Work supported by NASA-BioServe NAGW-1197 and NASA-NSCORT NAGW-2328.