# EFFECTS OF SILVER ION ($Ag^+$) ON A CELLULAR SHAPE CHANGE IN THE ABSENCE OF MICROTUBULES IN FERTILIZED EGGS OF *ILYANASSA OBSOLETA*

Gary W. Conrad and Diane C. Wells
Division of Biology, Kansas State University,
Manhattan, KS 66506-4901

Fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta* Stimpson [= *Nassarius obsoletus* Say] undergo a dramatic change in cell shape resembling cytokinesis before and during first cleavage. In this process, termed polar lobe formation, a microfilament-containing contractile ring forms a furrow, the polar lobe constriction, slightly below the cell equator that pinches the fertilized egg from a diameter of 166 um to a neck of less than 10 um over a period of 1 hr. We have shown previously (Conrad, A.H, et al. *Cell Motil. & Cytoskel.* 27:117-132, 1994) that silver ions ($Ag^+$) allow the polar lobe constriction process to begin at the same time as controls, but then to constrict around a bundle of microtubules. Controls, in contrast, form a constricted polar lobe neck around the same region of cytoplasm that normally, however, contains very few microtubules. A normal polar lobe neck remains constricted for only approximately 4 min. and then relaxes completely, whereas the polar lobe neck in a $Ag^+$-treated cell remains tightly constricted, eventually cleaving the cytoplasmic neck. Because in this model system the cleavage of a cytoplasmic neck by a constriction that is normally transient is correlated with the experimentally-induced presence of microtubules in a cellular domain normally devoid of them, we have heretofore hypothesized that treatment of the cells with $Ag^+$ caused, directly or indirectly, a stabilization of microtubules. Indeed, treatment of these cells with reference standard microtubule stabilizing agents, taxol and hexylene glycol, produced phenotypes remarkably similar to those from $Ag^+$ treatment: constriction of the cell down to a tight neck containing many microtubules, followed by cleavage of the neck (Conrad, A.H., et al. *J. Exptl. Zool.* 262:154-165, 1992; Conrad, A.H., et al.. *J. Exptl. Zool.* 269:188-204, 1994). These results suggested that the phenotypic effects seen in response to treatment of the cells with Ag+ required the [experimentally-induced] presence of microtubules. During the summer of 1997, we tested this hypothesis by determining the extent to which $Ag^+$ could interfere with a cellular shape change if microtubules were first removed experimentally.

Our previous work had demonstrated that, if microtubules are first caused to depolymerize experimentally by treatment of the cells with colchicine or nocodazole, formation of the polar lobe constriction begins at the same time as in controls, slowly constricting the cell at the same rate as controls (Phase I), but that constriction then ceases - neither constricting further (Phase II, in controls), nor relaxing (Conrad, G.W., and Williams, D.C. *Develop. Biol.* 36:363-378, 1974; Conrad, G.W., and Vernon, P.E. *Intl. J. Invert. Reprod. & Develop.* 9:195-207, 1986). Instead, such constrictions remain locked at a point of mid-constriction for hours, essentially blocked at the normal Phase I / Phase II transition point. We therefore asked whether $Ag^+$ could influence the process of polar lobe constriction, and the cessation of constriction, in such nocodazole-treated cells, using the same concentrations of $Ag^+$ and nocodazole as used in the published studies noted above.

Fertilized eggs were transferred to sea water containing nocodazole alone, $Ag^+$ alone, nocodazole + $Ag^+$, or control solution [seawater alone or sea water + DMSO (dimethylsulfoxide, solubilizing agent for nocodazole)]. Polar lobe formation began on schedule with controls in all solutions, and neck constriction progressed at the same rate in all control and experimental groups. However, when the Phase I / Phase II transition point was reached in controls, cells treated with nocodazole alone stopped constricting at that point, as previously described, but in cells treated with nocodazole + $Ag^+$ the polar lobe neck continued to constrict, eventually yielding cells with tightly constricted necks. Cells treated with $Ag^+$ alone formed cleavage furrows and abnormally long-lived, very tight, long, constricted polar lobe necks that eventually cleaved, as previously described. Cells from all groups were fixed for electron microscopy to allow comparison of their relative numbers of microtubules (these sections are still under analysis).

These results suggest that, whatever else $Ag^+$ may be doing by its direct or indirect effects on microtubules (or tubulin subunits), this metal ion may directly or indirectly affect the structure and function of other cytoskeletal components, such as F-actin or myosin, for example.

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