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Inhibitors of Protein Phosphatases (Okadaic Acid and Tautomycin) Block Sea Urchin Development

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Protein phosphorylation, regulated by protein kinases and phosphatases, is critical for cell growth, regulation, and development (1–3). Phosphatase inhibitors, which prolong the phosphorylated state of proteins, can often perturb cell development. Our laboratory has been interested in utilizing two of these compounds, okadaic acid, isolated from the marine sponge *Halichondria okadai*, and tautomycin, isolated from *Streptomyces spirover ticillatus*, to examine the role of phosphatases in sea urchin development. Previous work demonstrated that these compounds are effective inhibitors of serine and threonine phosphatases, in particular, phosphatases 1 (PP-1) and 2A (PP-2A) (4). Okadaic acid is more selective, preferentially inhibiting PP-2A, whereas tautomycin inhibits PP-1 and 2A with equal effectiveness (4). Recent studies indicate that PP-1 may be important in the initial responses of sea urchin eggs to fertilization (5). In the present studies we sought to determine whether okadaic acid and tautomycin could differentially modify fertilization and early development in the sea urchin *Arbacia punctulata*.

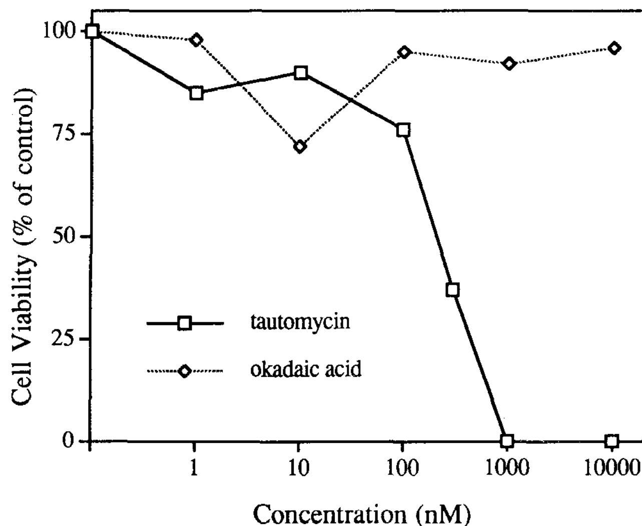

Figure 1. *Effects of phosphatase inhibitors on the viability of sea urchin embryos. Eggs obtained from* Arbacia punctulata *were fertilized* in vitro *in the presence of increasing concentrations of okadaic acid (broken line) or tautomycin (solid line). The percentage of viable embryos was calculated from the number of embryos that were freely rotating 24 h after fertilization.*

In initial experiments, sea urchin sperm and eggs were incubated with increasing concentrations of okadaic acid and tautomycin (1–1000 n*M*). We found that these compounds had no effect on sperm activation or on early events of fertilization (not shown). Thus, in all cases, >95% of the eggs raised fertilization membranes and formed embryos. We also found that blastula rotation, representing a later stage of differentiation, was selectively inhibited by tautomycin but not okadaic acid. Tautomycin was a potent inhibitor of the transition into the rotating blastula stage and was dose dependent at concentrations of 100–1000 n*M* (Fig. 1). Embryos treated with okadaic acid, however, did not exhibit this effect (Fig. 1). No embryos treated with tautomycin (100 n*M*–10 μM) developed into plutei (data not shown). Only very high concentrations of okadaic acid (1–10 μM) inhibited the hatching of plutei, consistent with the small effect of this compound on the initial development of motility.

In conclusion, tautomycin, but not okadaic acid, is an effective inhibitor of sea urchin development, acting on the maturation into rotating blastula. We speculate that the preferential inhibition of PP-1 by tautomycin may reflect differential roles of PP-1 and PP-2A in this process.

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Reversible Regression of Cytokinesis Induced by Ca^{2+} Ionophore

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Transient changes in intracellular free Ca^{2+} concentration are spatially and temporally coupled to specific cell cycle events such as mitosis and cytokinesis (*e.g.*, 1, 2). In the sea urchin embryo, an endogenous rise in Ca^{2+} precedes cytokinesis (3); and in the medaka embryo, this transient Ca^{2+} increase is localized to the cleavage furrow (4). Injection of Ca^{2+} chelators into the sand dollar embryo prior to cytokinesis arrests development and inhibits furrow formation (5). We have examined

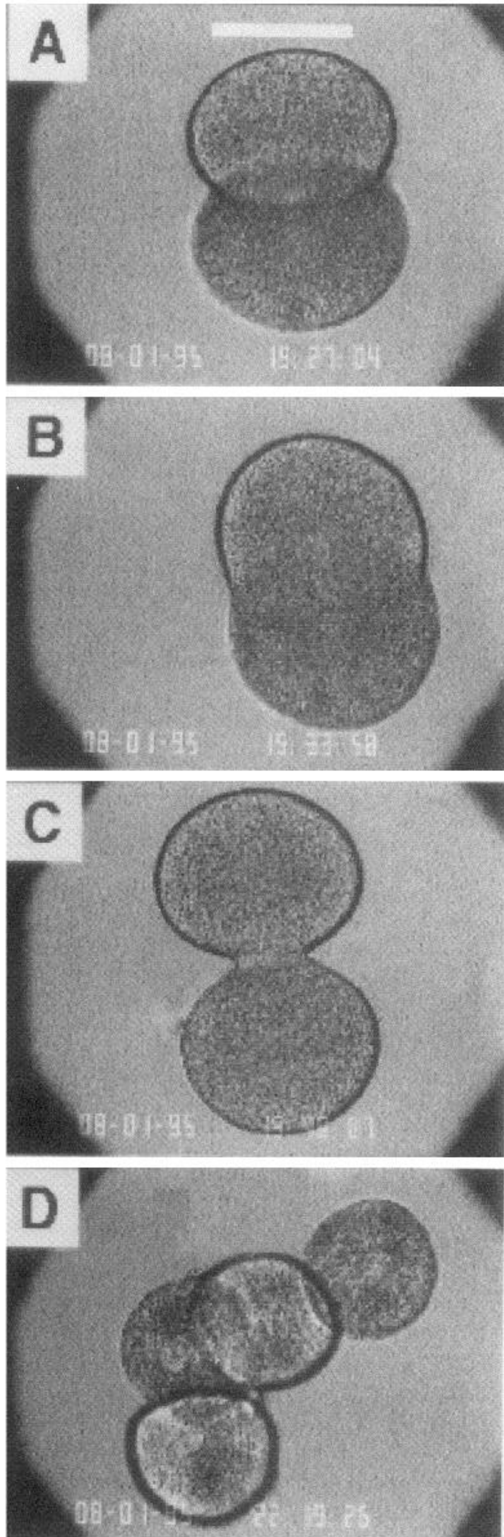

Figure 1. Lytechinus variegatus *embryo at early stages of development imaged with polarized light microscopy. (A) Embryo at first cell division cycle, with cleavage furrow, at the time of A23187 perfusion. (B) Cleavage furrow regression induced by A23187. (C) Cytokinesis resumed at the same site when the A23187 was washed out with ASW. (D) The same embryo continued through cell division and development at the next cycle after a single A23187 treatment and ASW washing. Bar = 50 μm.*

further the roles that Ca^{2+} plays in cytokinesis, specifically downstream of cleavage furrow formation; our approach was to induce a global Ca^{2+} increase within the sea urchin embryo.

Lytechinus variegatus eggs and sperm were collected by electrically stimulating the sea urchins. The eggs were fertilized and the embryos developed in artificial seawater (ASW) containing about 10 m*M* Ca^{2+}. The fertilization envelope was removed from the fertilized eggs by washing with a digestion mix containing 1 m*M* DTT and 15 μg/ml pronase at pH 8.9. The eggs were then place in a wedge-profiled perfusion chamber and observed with polarized light microscopy.

At 18°C, the fertilized sea urchin eggs completed the first cell division in ~2 h, with the cleavage furrow appearing at ~1:45 h. After the first few minutes of furrow formation, we perfused the eggs with ASW containing 5 μg/ml A23187, a Ca^{2+} ionophore and an effective transporter that presumably causes a global influx of extracellular Ca^{2+} into the dividing egg.

Figure 1A shows a sea urchin egg with cleavage furrow just before A23187 perfusion. Furrow progression in the egg immediately stopped and the furrow regressed outwardly (Fig. 1B) until the egg returned to its original spherical shape. This furrow regression was reversible, and cytokinesis resumed *at the same site* when the A23187 was washed out with ASW within 10–20 min of the initial A23187 perfusion (Fig. 1C). The cycle of cleavage furrow regression in A23187 followed by the resumption of cytokinesis after washing could be repeated two to three times with the same cell. After two cycles of A23187 application and ASW washing, the embryo continued to divide and develop. Cells exposed to 5 μg/ml of A23187 for more than 15–20 min did not survive the treatment.

To our knowledge, this is the first example of a reversible regression of cytokinesis induced by Ca^{2+} ionophore A23187. A previous study by Arnold (6) showed that A23187 enhances furrowing in the squid embryo, but the concentration of ionophore used in those experiments was not stated. In our study, the reversible regression of cytokinesis was dependent on temperature, extracellular Ca^{2+} concentration, ionophore concentration, stage of the cell cycle, and ionophore exposure time. We have not yet determined the exact mechanism underlying the reversible regression of cytokinesis induced by A23187. However, we propose that A23187 causes a global increase in free Ca^{2+} that may activate actin severing proteins or deactivate actin cross-linking proteins; or alternatively, Ca^{2+} modulates binding of myosin to actin filaments directly or via a Ca^{2+} sensitive kinase such as myosin light chain kinase or protein kinase C.

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