How-To-Do-It

The Sea Urchin Embryo: A Remarkable Classroom Tool

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The experimental material of choice for the investigation of many developmental mechanisms is the sea urchin embryo (Giudice 1986; Davidson, et al. 1982; Oppenheimer & Lefevre 1989). It is the organism of choice because of specific qualities that also make it ideal for classroom use. Unlike chick embryos, which are covered by shells and available in small numbers, and unlike frogs, whose males are often sacrificed to obtain sperm and females are hormonally induced to ovulate, sea urchin embryos are available by the billions and clearly display embryonic development. No shells are present to block viewing and all experiments are done in the simplest of media—natural or artificial sea water. And, teachers can easily obtain sea urchins.

Sea urchin embryos have been used for decades in the classroom and research laboratory and are the finest tools available for introducing students to the wonders of embryonic development and the world of research science. This article will illustrate how the sea urchin has been used to uncover key developmental mechanisms and how it can be used in the classroom to excite the students' curiosity and facilitate their introduction to well defined research experiences.

The Sea Urchin in Research

Many important discoveries in the areas of fertilization and early development resulted from experiments with sea urchin embryos. One such exciting discovery is the story of egg activation.

How does a tiny sea urchin sperm that is only 0.0002 percent of the egg surface trigger the multitude of changes that occur in the fertilized egg? The sea urchin has been most instrumental in answering this question. Within three seconds after the sperm binds to the sea urchin egg, a membrane potential change occurs (Whitaker & Steinhardt 1985). By 30 seconds, calcium ions begin to be released from the endoplasmic reticulum to a free state in the cytoplasm, followed by the cortical reaction, in

which cortical granules that line the inner surface of the egg plasma membrane begin to fuse with the membrane, releasing their contents. This leads to the formation of the fertilization membrane that blocks the entry of additional sperm (Whitaker & Steinhardt 1985; Oppenheimer & Lefevre 1989) (Figure 1).

A variety of elegant experiments have led to the key finding that free calcium ions are directly responsible for the cortical reaction. Steinhardt, Epel, Chambers, Pressman and Rose used a substance called calcium ionophore A23187, which causes release of stored calcium in cells, duplicating some of the events occurring shortly after sperm binding. They found that many of the same events that occurred after sperm binding also occurred with the use of this chemical in the absence of sperm. This suggested that calcium ions must play a key role in egg activation (Steinhardt & Epel 1974; Whitaker & Steinhardt 1985).

This suggestion was strengthened by Victor Vacquier's experiments at the Scripps Institution of Oceanography. Sea urchin eggs were bound to glass slides and lysed, exposing the inner membrane surface to which the cortical granules are attached. The slides were exposed to a variety of salt solutions. Only calcium ions caused fusion of the cortical granules with the plasma membrane in much the same way as in the intact egg during the cortical reaction. Experiments such as these, using sea urchin material, have been instrumental in helping us to understand some of the events that occur during fertilization (Vacquier & Epel 1978).

Recent work with the sea urchin system has helped explain exactly how sperm cause egg activation. The binding of sperm to the egg cell membrane receptor appears to change the conformation of the receptor, which activates a GTP-binding protein (G-protein) (Turner, et al. 1986). This protein then activates phospholipase C, which in turn splits phosphatidylinositol 4, 5 bisphosphate into diacylglycerol and inositol trisphosphate (IP₃). IP₃ causes the endoplasmic retic-

ulum to release calcium ions, which in turn cause the cortical reaction to occur. Diacylglycerol activates protein kinase C, which stimulates the sodium/hydrogen pump to pump hydrogen ions out of the egg and sodium ions in, resulting in an increased intracellular pH. This rise in pH, along with the free calcium ions, appears to be instrumental in activating protein synthesis and DNA replication (Berridge 1985; Swann & Whitaker 1986; Cipa & Whitaker 1986; Whitaker & Irvine 1984; Busa, et al. 1985; Gilbert 1987). Figure 1 summarizes the proposed causative events in sea urchin egg activation.

The early embryo now cleaves and develops into the hollow ball stage (blastula). This is followed by the gastrula, a stage in which many dramatic changes occur. The well known embryologist Lewis Wolpert is widely believed to have said that it is not birth, marriage, or death, but gastrulation which is truly the most important time in your life. During gastrulation, the embryo begins to take shape. Without this process, many organisms would be round little balls that could never amount to anything.

The sea urchin embryo, because of its simplicity and transparency, has

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been helpful in understanding gastrulation mechanisms. Investigators have been able to observe how the cells behave during gastrulation. Small cells, called micromeres, lose adhesive affinity with other cells in the vegetal plate region of the blastula. They migrate into the central cavity—the blastocoel—and are called primary mesenchyme cells. These cells migrate along the extracellular matrix in the blastocoel by tenaciously adhering to fibronectin, a large glycoprotein secreted by blastula cells (Wessel, et al. 1984; Fink & McClay 1985) that appears to control their migration (Katoh

& Hayashi 1985). A variety of experiments in which synthesis of certain sulfated glycoproteins was inhibited or assembly of microtubules prevented suggests that these components also play important roles in mesenchymal cell migration (Karp & Solursh 1974; Anstrom, et al. 1987; Gibbins, et al. 1969).

In some sea urchin species, projections, called filopodia, which extend from secondary mesenchyme cells at the advancing tip of the primitive gut (archenteron), stick to the inner surface of the blastocoel wall and contract, helping to complete the forma-

tion of the elongated archenteron (Trinkaus 1984).

These are a few of the advances in developmental biology discovered through experiments with sea urchin embryos. Sea urchins can also be used successfully in the classroom.

Classroom Experiments

Sea urchin kits containing all the materials and instructions for experiments for 600 or more students are available from companies such as Pacific Biomarine Laboratories (P.O. Box 1348, Venice CA 90294) at a cost of

Figure 1. Model showing proposed causative sequence of some events occurring during egg activation in sea urchin fertilization.

about \$110 (which includes shipping by air express). When the kit arrives, the sea urchins can be used immediately or stored in a refrigerator as packed for up to a few days. After gametes are removed from the sea urchins by inoculating them with 0.5 M potassium chloride (included in the kit), the undiluted sperm and the eggs diluted in sea water can be used immediately or stored for up to a few days in the refrigerator. Long term maintenance of adult sea urchins is best done in refrigerated marine aquaria at 9-12°C.

Large groups of students can be introduced to sea urchin fertilization by placing a small drop of eggs on a slide. As the student views the eggs under the microscope, a drop of freshly diluted sperm (0.1 ml undiluted sperm added to 10 ml sea water) is added and fertilization can be clearly viewed. A discussion of the events that occur during fertilization, as presented earlier (Figure 1), can provide the students with a feeling for what is going on right before their eyes.

Early development can also be beautifully observed by students using this system (Figure 2). Fertilize the diluted eggs (1 ml settled eggs in 100 ml of sea water) in a large beaker

with freshly diluted sperm. Allow the eggs to settle out; pour off the sea water/sperm suspension and refill with fresh sea water (natural or artificial). Pour the diluted zygotes into plastic Petri dishes until the dishes are half full and store them in a cool room (15-17°C is best). In a couple of hours the zygotes will undergo cleavage and in about a day the blastula stage embryos will hatch out of their fertilization membranes. Gastrulation follows.

Student Research

With the introductory exercise behind them, students are generally so intrigued with this living system that they are eager to do more. This system provides an ideal opportunity to introduce them to personal research projects. For nearly two decades we have been using these sea urchin fertilization and development exercises as an introduction to student research in both pre-college and college programs. These programs have received widespread recognition through NSF grants, an NIH grant, Thomas Eckstrom Trust and Joseph Drown Foundation grants, NASA grants and fellowships and awards from the Trustees of the California State Uni-

versity system, American Cancer Society and California Science Teachers Association.

What is so great about using the basic sea urchin exercise in class is that offshoots are easily accomplished in minutes. First I describe possible projects that students can easily do in the classroom using little more than sea urchin gametes and artificial sea water. For example, student projects can involve changing salt concentration of sea water, changing specific ions or adding chemicals, changing the pH or temperature of the sea water and observing the effects of these changes on fertilization or early development. You can use such criteria as counting percent of eggs with fertilization membranes or abnormalities observed during development compared with normal control conditions.

These experiments are so simple to do and the results so easy to obtain that generally 100 percent of the students in a class are able to successfully carry out one of these mini-projects. We require that a brief experimental plan with background references first be submitted to the instructor who then makes suggestions and returns it to the students. Students make up their solutions during one class period and conduct the experiments during the next couple of days. Upon completion of the project, students write up their experiments according to standard format found in science journals and follow it up with an oral presentation of their work. This approach generally works best when students work in groups of three or four where each student has specific tasks to accomplish. The group setting improves self confidence and leads to a high degree of mutual assistance, as has been found in other learning situations (Lapp, et al. 1989; Johnson & Johnson 1975).

Our students are working on some very successful projects, as are teachers participating in our National Science Foundation-sponsored teacher enhancement program. A high school student who has been working with us for two years studied the effects of direct electric current on fertilization and early development in the sea urchin. He won a best paper finalist award at the Southern California Academy of Sciences annual meeting. Another student has been awarded an \$18,000-a-year fellowship from NASA to work with us on a computer analysis of the parameters affecting sea urchin fertilization and early development. This will prepare

Figure 2. Cleavage and gastrulation in the sea urchin embryo.

the sea urchin system for study under zero gravity conditions in space.

Scores of students in our laboratory are studying the molecular mechanisms involved in controlling cell adhesion in the sea urchin embryo (with support over the years from NSF, NIH, NASA, Thomas Eckstrom Trust, Joseph Drown Foundation, CSU Foundation, Northridge Student Projects Committee and CSUN Research and Grants Committee). In these experiments, students grow sea urchin embryos to the swimming blastula stage (about 23 hours for the sea urchin *Strongylocentrotus purpuratus*), then disaggregate them into viable single cells by incubating the embryos in calcium-magnesium-free sea water. When the cells are returned to normal sea water that contains calcium and magnesium, they reaggregate to form swimming embryo-like structures called embryoids, which can undergo further development. The students therefore learn that by simple experimental manipulations, embryos can be taken apart and put back together.

Our students use this intriguing concept to study the molecules required for cell adhesion, a property that is essential for normal embryonic development and which, when defective, plays a key role in the spread of cancer. They incubate the single sea urchin embryo cells with a variety of substances, some isolated from living sea urchin embryos, to test their effects on the ability of the cells to reaggregate. These simple student experiments have led to the discovery of specific proteins and sugars that appear to be involved in mediating adhesion in this system (Asao & Oppenheimer 1979; Oppenheimer & Meyer 1982a, 1982b).

Many students who were introduced to the sea urchin in our classes are now research scientists and have been co-authors of our research publications (28 of our research publications include 72 student co-author citations). The sea urchin embryo is so easy to work with and so likely to yield meaningful results that we believe it can't be beaten as a tool to introduce students to the excitement of experimental biology.

Full page-sized copies of the figures included in this article and other teaching aids for classroom use can be obtained free of charge by contacting the author.

References

Anstrom, J.A., Chin, J.E., Leaf, D.S., Parks, A.L. & Raff, R.A. (1987). Localization and expression of msp 130, a pri-

mary mesenchyme lineage-specific cell surface protein of the sea urchin embryo. *Development*, 101, 255-265.

Asao, M.I. & Oppenheimer, S.B. (1979). Inhibition of cell aggregation by specific carbohydrates. *Experimental Cell Research*, 120, 101-110.

Berridge, M.J. (1985). The molecular basis of communication with the cell. *Scientific American*, 253, 142-152.

Busa, W.B., Ferguson, J.E., Joseph, S.K., Williamson, J.R. & Nuccitelli, R. (1985). Activation of frog (*Xenopus laevis*) eggs by inositol triphosphate. I. Characterization of Ca^{2+} release from intracellular stores. *Journal of Cell Biology*, 100, 677-682.

Ciapa, B. & Whitaker, M. (1986). Two phases of inositol polyphosphate and diacylglycerol production at fertilization. *FEBS Letters*, 195, 347-351.

Davidson, E.H., Hough-Evans, B.R. & Britten, R.J. (1982). Molecular biology of the sea urchin embryo. *Science*, 217, 17-26.

Fink, R.D. & McClay, D.R. (1985). Three cell recognition changes accompany the ingress of sea urchin primary mesenchyme cells. *Developmental Biology*, 107, 66-74.

Gibbins, J.R., Tilney, L.G. & Porter, K.R. (1969). Microtubules in the formation and development of the primary mesenchyme of *Arbacia punctulata*. *Developmental Biology*, 18, 523-539.

Gilbert, S.F. (1987). *Developmental biology* (2nd ed.). Sunderland, MA: Sinauer.

Giudice, G. (1986). *The Sea Urchin Embryo*. Boston: Springer Verlag.

Johnson, D. & Johnson, R. (1975). *Learning together and alone: Cooperation, competition and individualization*. Englewood Cliffs, NJ: Prentice Hall.

Karp, G.C. & Solursh, M. (1974). Acid mucopolysaccharide metabolism, the cell surface, and primary mesenchyme cell activity in the sea urchin embryo. *Developmental Biology*, 41, 110-123.

Katoh, H. & Hayashi, M. (1985). Role of fibronectin in primary mesenchyme cell migration in the sea urchin. *Journal of Cell Biology*, 101, 1487-1491.

Lapp, D., Flood, J. & Thorpe, L. (1989).

Cooperative problem solving, enhancing learning in the secondary science classroom. *The Science Teacher*, 51, 112-115.

Oppenheimer, S.B. & Lefevre, G. (1989). *Introduction to Embryonic Development* (3rd ed.). Boston: Allyn and Bacon.

Oppenheimer, S.B. & Meyer, J.T. (1982a). Isolation of species-specific and stage-specific adhesion promoting component by disaggregation of intact sea urchin embryo cells. *Experimental Cell Research*, 137, 472-476.

Oppenheimer, S.B. & Meyer, J.T. (1982b). Carbohydrate specificity of sea urchin blastula adhesion component. *Experimental Cell Research*, 139, 451-455.

Steinhardt, R.A. & Epel, D. (1974). Activation of sea urchin eggs by a calcium ionophore. *Proceedings of the National Academy of Sciences, U.S.A.*, 71, 1915-1919.

Swann, K. & Whitaker, M. (1986). The part played by inositol trisphosphate and calcium in the propagation of the fertilization wave in sea urchin eggs. *Journal of Cell Biology*, 103, 2333-2342.

Trinkaus, J.P. (1984). *Cells into organs: The forces that shape the embryo* (2nd ed.). Englewood Cliffs, NJ: Prentice Hall.

Turner, P.R., Jaffe, L.A., & Felin, A. (1986). Regulation of cortical vesicle exocytosis in sea urchin eggs by inositol 1,4,5-trisphosphate and GTP-binding protein. *Journal of Cell Biology*, 102, 70-76.

Vacquier, V.D. & Epel, D. (1978). Membrane fusion events during invertebrate fertilization. In G. Poste & G.L. Nicolson (Eds.), *Membrane fusion*. New York: Elsevier.

Wessel, G.M., Marchase, R.B. & McClay, D.R. (1984). Ontogeny of the basal lamina in the sea urchin embryo. *Developmental Biology*, 103, 235-245.

Whitaker, M. & Irvine, R.F. (1984). Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature*, 312, 636-639.

Whitaker, M.J. & Steinhardt, R. (1985). Ionic signalling in the sea urchin egg at fertilization. In C.B. Metz & A. Monroy (Eds.), *Biology of Fertilization* (Vol. 3, pp. 167-221). Orlando: Academic Press.

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