Derivation of cell lines from *Strongylocentrotus droebachiensis*, the Northern sea urchin

Christopher J. Bayne[1] and Angela Parton[2].

[1]Department of Zoology and Marine and Freshwater Biomedical Science Center, Oregon State University, Corvallis, OR 97330
[2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

The value of permanently growing cell lines from model organisms is widely recognized. A persistent absence of cell lines from non-arthropod invertebrates has stymied research on many valuable species[1,2,4]. Among the tens of thousands of such species, a few - the nematode *Coenorhabditis elegans* and several marine molluscs (*e.g.*, oysters), echinoderms (sea urchins) and tunicates (sea squirts) for example - have grown in importance as models for biomedically relevant research. To provide tools for pharmacological, toxicological, disease-related, functional genomic and other research, repeated attempts have been made to establish cell lines from these animals. At MDIBL, approaches based on Japanese research[3] have enabled the establishment of a cell culture system for *Strongylocentrotus droebachiensis,* an urchin whose West Coast congener *S. purpuratus* is the subject of a genome sequencing project. Of 6 tissues whose cells were placed in culture, two (Polian vesicle and axial organ) yielded long-term survivors that began to proliferate *in vitro*. These were passaged and split. Progeny from these cultures are expected to serve as tools for a diverse array of studies. Other cultures yielded thraustochytrid protists that are common parasites of marine invertebrates worldwide.

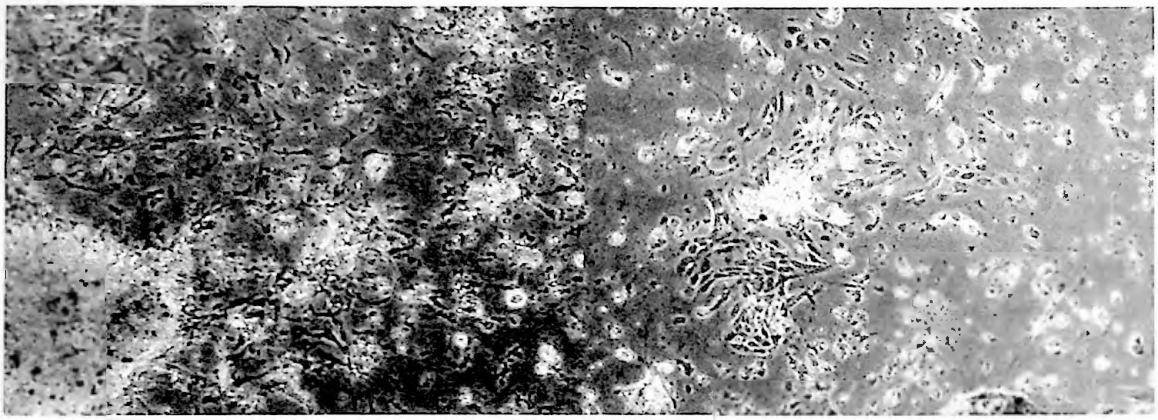
The waters around the MDIBL support a healthy population of *S. droebachiensis*. As sedentary invertebrates that dwell in the intertidal and immediate sub-tidal zones, these animals meet criteria as excellent sentinels of environmental contaminants and thus are species of choice for aquatic toxicology research. Furthermore, like humans, these organisms are deuterostomes. Therefore, their genomes comprise valuable sources of information on the ancestral status of human genes at a crucial evolutionary juncture.

A mixture of L-15 medium, DME and F-12 (5:3.5:1.5, called LDF) was prepared, and one volume was diluted with 4 volumes of filtered sea water and sterile filtered. With the addition of antibiotics (penicillin 200 U/ml, streptomycin 200 µg/ml, ampicillin 25 µg/ml), sodium bicarbonate at 0.18 g/L and HEPES at 15 mM, this comprised the basic Urchin medium. Individual urchins were prepared for surgery by being passed through two sterile filtered sea water (f-SW) baths (10 min each), followed by 30 min in f-SW containing Zonker - a powerful mixture of four antibiotics. Zonker, added at 1 part in 10, contains penicillin G 7.8 g, streptomycin sulfate 12.5 g, neomycin sulfate 12.5 g, Bacitracin 1.78 g in 50 mL Hanks balanced salt solution. The animal was removed from the bath and allowed to drain for a minute in a sterile hood. Scissors were then used to separate its upper and lower halves. Sterile iris scissors and fine forceps were used to separate target tissues (see below) from neighboring tissues, and each collected tissue was transferred to the first of two baths of Urchin medium containing antibiotics (10% Zonker). Media and tissues were kept cold during processing.

After the second wash, tissue pieces were minced with iris scissors. The product was washed by centrifugation, the supernatant was replaced with fresh Urchin medium with 10% Zonker and experimental additives, and the pellet was resuspended and used to inoculate a T-25 culture flask and wells of a 24-well plate. Culture vessels were held at 20°C in ambient air.

Additives included fetal calf serum (heat inactivated) at 1-3%; insulin at 10 µg/ml; transferrin at 10 µg/ml; fibroblast growth factor at 50 ng/ml; epidermal growth factor at 50 ng/ml; β-mercapto-ethanol at 55µM; chemically defined lipid supplement at 1 µl/ml; selenium at 10 nM, and L-glutamine at 200 µM. After one day, the level of Zonker was reduced to 1% by dilution using Zonker-free LDF.

Cultures were initiated with coelomocytes, axial organs, Polian vesicles, ampullae, podia, and muscles from the aboral surface of Aristotle's lantern. Axial organ and Polian vesicle cells survived long term (5 months at time of writing) and have proliferated *in vitro* (Figures 1A, 1B). These cultures were passaged once, split 1:2, and proceeded to grow again to confluence. Confirmation of their species of origin will be sought using sequence data available in GenBank.



A B

Figure 1. *S. droebachiensis* cells that have survived long term and begun to proliferate *in vitro*. A: Cells from Polian vesicles. B: Cells from an axial organ.

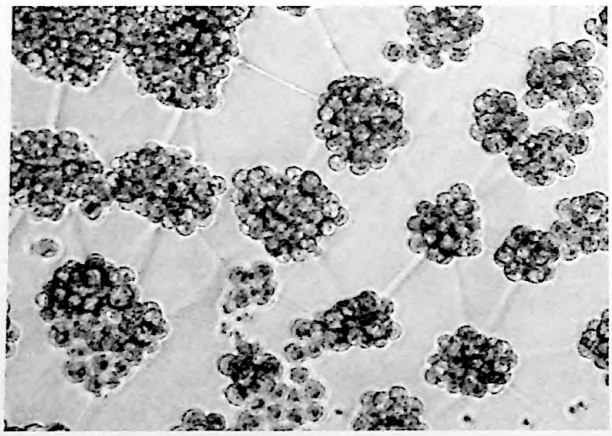


Figure 2. In several cultures, cells resembling these grew. They were likely to have been thraustochytrid protists.

The success of this project destroys an enduring myth of some unknown block to the propagation of cells from non-arthropod marine invertebrates[1,4] and gives hope for successful establishment of cell lines from other medically-relevant species.

Funds were kindly provided through a New Investigator Award from MDIBL to Christopher Bayne and a grant from the NIH: P30 ES-03828. We thank David Barnes for helpful advice and encouragement. He and Angela Parton are members of the MDIBL Membrane Toxicity Center (MFBSC).

1. Bayne, C.J. Breaking the barriers to continuous long-term cell propagation: freshwater mollusks. NOAA Technical Memorandum NMFS-F/NEC-98. pp 13-15, 1993.
2. Bayne, C.J. Invertebrate Cell Culture Considerations: Insects, Ticks, Shellfish, and Worms. Chapter 10, pp 187-201 *In* Methods in Cell Biology, 57. D.W. Barnes & J.P. Mather, editors. Academic Press. 1998.
3. Kawamura, K., and Fujiwara, S. Establishment of cell lines from multipotent epithelial sheet in the budding tunicate, *Polyandrocarpa misakiensis. Cell Struct Func.* 20: 97-106, 1995.
4. Rinkevich, B. Cell cultures from marine invertebrates: obstacles, new approaches and recent developments. *J. Biotechnol.* 70: 133-153, 1999.