Long-term cultures of cells from *Strongylocentrotus purpuratus* and *Schistosoma mansoni*.

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Selection of the echinoderm *Strongylocentrotus purpuratus* as a model species for genomic sequencing has enhanced the need for cell lines from this species. Previously[2], we determined that several tissues from the closely related, locally available urchin *S. droebachiensis* could be placed in culture, and the cells would multiply. Interrogation of the public databases for putative growth factors (GF) and GF receptors expressed in this genus allowed us to identify several GFs that were commercially available and might be evaluated for their ability to provoke cell proliferation. Therefore, in September 2004, six specimens of *S. purpuratus* were brought from their native Oregon to MDIBL as sources of cells for *in vitro* culture.

Earlier we had also determined protocols and conditions that are well suited for the long term culture of cells of the human blood fluke, *Schistosoma mansoni*[1], but we had been unable to surmount difficulties inherent in provoking these cells to proliferate *in vitro*. As *S. mansoni* continues to take a severe toll on human health in over 70 countries, and as knowledge of genetic sequences are accumulating rapidly for this species, derivation of cell lines from this species remains a highly desired goal. Consequently, several thousand sporocyst larvae of *S. mansoni* were also transported from Oregon to MDIBL in September, 2004. As with the urchin cells, we added putative GFs and some additional receptor ligands to the media used to maintain the cells. These molecules included insulin, transferrin, basic fibroblast GF, epidermal GF, acetyl choline, activin-A, ciliary neurotrophic factor, glial-derived GF, heregulin, insulin-like GFI and II, interleukin-6, interleukin-7, interferon-γ, neuropeptide Y, hepatocyte-derived GF, osteopontin, progesterone, bone morphogenetic protein-4, bone morphogenetic protein-5, thyroid hormone (T3), thyroglobulin and γ-amino butyric acid.

Drawing on the extensive cell culture resources that are uniquely available in the Center for Marine Functional Genomics Studies at MDIBL, we established primary tissue cultures from both the urchin and the blood fluke, and cells have been sustained in a healthy, germ-free state for 4 months. Major departures from conditions used previously included the use of nitrogen gas to create an hypoxic atmosphere for the schistosomes, the use of media that have lowered levels of organic components, and the addition to media (for both species) of growth factors and other biologically active molecules which, from knowledge of cell biology and of the transcriptomes of these 2 species, could be expected to have growth-promoting activities. Mining of DNA sequence databases for *Strongylocentrotus* and *Schistosoma* was the method used to select the molecules for these tests.

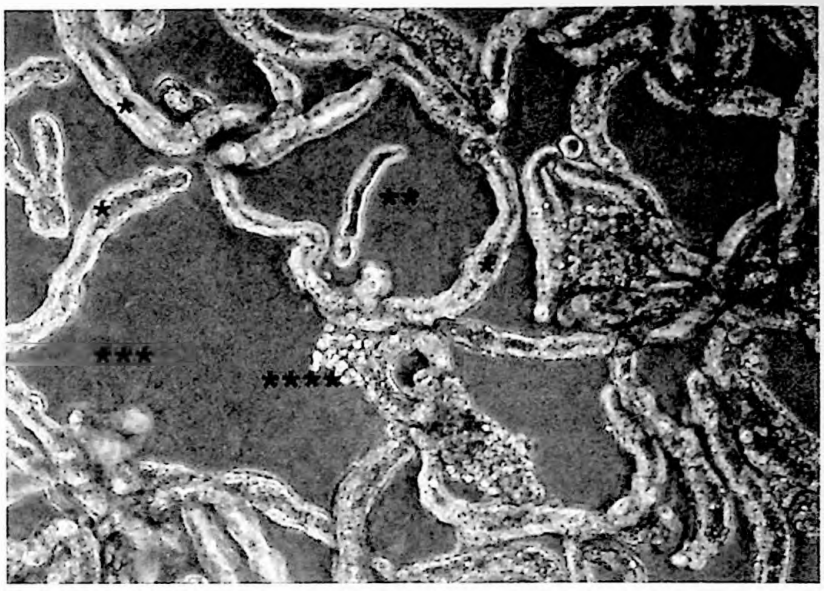

Fig. 1. Sporocysts of *Schistosoma mansoni* after 3 weeks in culture. Many mother* sporocysts and one of their smaller daughters** are present. The tegument has produced extensions*** into the growth space around some sporocysts. A cluster of bright cells****, thought to be germinal cells that have migrated from the internal cavity of a sporocyst, is seen towards the center of the photomicrograph.

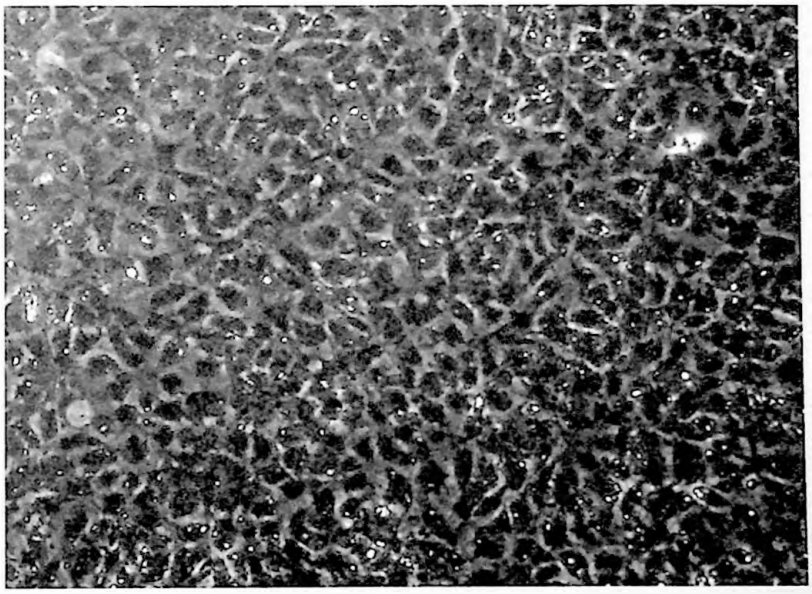

Fig. 2. Cells derived from tissues located between the lateral faces of the main teeth of Aristotle's lantern of *Strongylocentrotus purpuratus*. These cells have the appearance of cells that will proliferate *in vitro* once they have access to required growth factors that are either provided by the investigator or synthesized by the cells themselves.

As illustrated in Figures 1 and 2, both projects were fruitful. It is the nature of cell line development to require several weeks to several months in order to obtain indefinitely propagating cell lines. Consequently, it is not to be expected that a cell line will be obtained in the course of a month. After up to one month of intense husbandry (on the departure of the first author from MDIBL), the cultures were placed on a maintenance regime. At the time of writing, both urchin and schistosome cells remain viable. No explicit data on DNA synthesis or cell proliferation has been sought. However, cells of the type shown in Figure 2 have been passaged successfully. The ability to maintain healthy cell cultures over such a long term is fundamental to the development of permanent lines. It is anticipated that further experimental manipulations of growth conditions including media additives and manipulation of the physical environment will be evaluated as means to provoke cell multiplication in, and derivation of cell lines from, these cultures.

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1. **Bayne, C.J.** and **Barnes, D.W.** Culture of cells from two life stages of *Schistosoma mansoni*. *Cytotechnology* 23:205-210. 1997.
2. **Bayne, C.J.** and **Parton, A.** Derivation of cell lines from *Strongylocentrotus droebachiensis*, the Northern sea urchin. *Bull. Mount Desert Biological Laboratory*. 43: 62-64, 2004.