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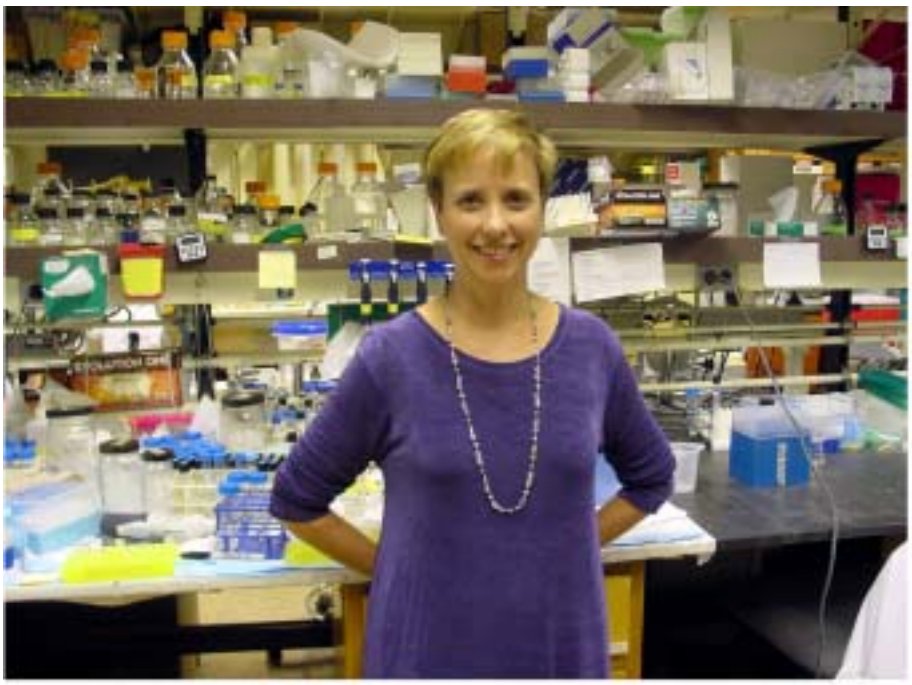
Searching for New Anti-Cancer Drugs: Biosynthesis of Bacteria-Produced Bryostatins

Margo Haygood
University of California, San Diego—Scripps Institution of Oceanography

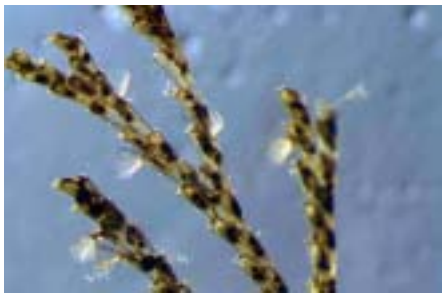
In the search for new medicines, no realm of the globe holds more intrigue than that of the sea. Many of the more complex, novel and interesting compounds discovered in the last decade have been extracted from marine organisms—typically soft corals and sponges, marine algae, even bacteria. Yet, despite the sea's promise, there remain major hurdles to developing commercially viable new marine-derived drugs. A leading one is that marine organisms often contain extremely low concentrations of a desired compound. Because these organisms may also be very rare or part of a fragile ecosystem that would be damaged by harvesting, gathering enough of a compound for extensive laboratory testing can be impossible. Still other animals, even if they are common, may be extremely difficult to collect.

As a result, pharmaceutical and academic research efforts remain focused on modifying or synthesizing compounds extracted from abundant forms of terrestrial life. To better harness the riches of the sea, Sea Grant is supporting research that will make it possible to "grow" rare marine compounds in sufficient quantities for conducting the requisite battery of laboratory tests.



With Sea Grant funding, Dr. Margo Haygood has developed techniques that may make it possible to produce marine natural products in nonmarine, easy-to-grow organisms. Photos: Scripps Institution of Oceanography.

This moss-like clump is actually a colonial marine animal, a bryozoan from which an anti-cancer compound has been extracted.



A microscopic view of branches of the bryozoan, *Bugula neritina.*

The Project

In this project, marine biology professor Dr. Margo Haygood of Scripps Institution of Oceanography was funded to investigate what could lead to a commercially viable method for producing large quantities of the patented compound Bryostatin 1 in easy-to-culture bacteria or yeasts, such as those used in brewing beer.

Bryostatin 1 is one in a family of macrocyclic lactones called bryostatins. Bryostatins are found in a brown moss-like marine organism, a bryozoan of the species *Bugula neritina*. Since the 1980s, people have recognized the strong anti-cancer properties of these compounds and have speculated that bacterial symbionts are involved in their production. The compounds are of added interest because, unlike many cancer treatments, they do not kill cancer cells directly but

instead inhibit the normal functioning of key proteins during cell replication, causing rapidly dividing cells to effectively commit suicide. None of this, however, has been parlayed into actually treating human disease.

In this project, Haygood has conducted a series of experiments that may make it possible to unlock the treasure chest of marine biological diversity to drug discovery. She has provided compelling evidence that bacteria living within *B. neritina* are indeed the source Bryostatin 1. Among the facts that point to this conclusion, *B. neritina* were observed to contain smaller amounts of bryostatin after being treated with antibiotics.

In other experiments, Haygood identified the genes that code for the production of bryostatins and then showed that these genes are expressed in only one bacterium, *Candidatus Endobugula sertula*.

This bacterium, experiments have shown, is transmitted from adult bryozoans to their larvae. This vertical, parent-child, transmission strongly suggests that the bryozoans benefit from the symbiosis. It has been hypothesized that bryostatins might deter predation, especially of larvae.

Because the bacterium needs to be mass produced to produce large enough bryostatin samples for testing, the lab attempted to culture *E. sertula*. When these attempts failed, Haygood's research took a second tack, that of sequencing the genes in the bacteria believed to be involved in bryostatin production.

This gene cluster proved to be gigantic, about 55,000 base pairs long, as long as some viruses.

In what scientists call pioneering research, Haygood and her lab were able to insert smaller segments of this gene cluster into different bacteria. In ongoing research, she and graduate students are trying to insert the entire gene cluster into a single bacterium. The goal is to find a bacterium whose genetic apparatus will accept the cluster and then synthesize the proteins that in turn produce bryostatins. If successful, it would be the first time researchers have been able to produce scarce marine compounds in nonmarine bacteria. Ideally, the scientists hope to insert the genes into a bacteria such as streptomyces, which is used extensively in industry to make antibiotics. With outside funding, her lab is continuing its efforts to culture *E. sertula* and is looking at the bioactivity of compounds from close relatives of *B. neritina.*

In their efforts to develop a method for producing marine natural compounds in commercial quantities, Haygood and her lab have made more progress than any other research group. The scientific community has been truly excited by her progress in developing a viable "supply technology" for marine natural products.

Applications

Haygood's work has led to a U.S. patent. The licensing rights have been bought by CalBioMarine Technologies, Inc. in Carlsbad, California.

Bryostatin is now in clinical trials for use in humans.

The results from this project have led to continuing research supported by the National Cancer Institute and the Department of Defense Breast Cancer Research Program.

Cooperating Organizations

CalBioMarine Technologies

Publication

Davidson. S.K., S.W. Allen, G.E. Lim, C.M. Anderson, and M.G. Haygood. 2001. Evidence for the biosynthesis of bryostatins by the bacterial symbiont *Candidatus Endobugula sertula* of the bryozoan *Bugula neritina*. *Appl. Env. Microbiol.* 67:4531–4537.

For more information:
Dr. Margo Haygood
Marine Biology Research Division
Scripps Institution of Oceanography
Tel.: (858) 534-5987
Email: mhaygood@ucsd.edu

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California Sea Grant College Program
Russell A. Moll, Director • Dolores M. Wesson, Deputy Director • Paul Olin, Interim Associate Director for Extension • Marsha Gear, Communications Director
University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0232
Phone:(858) 534-4440 Fax: (858) 453-2948 Web site: http://www-csgc.ucsd.edu