AQUACULTURE

Primordial Germ Cell Cultures for Transgenic Fish Production

Final Report

Objectives

The long-term goal of this project is to develop a cell-mediated gene transfer approach to the production of transgenic fish. To accomplish this goal, we are working to establish methods for the culture of fish primordial germ cells (PGCs). The PGC cultures will provide an efficient method to introduce foreign DNA into the fish genome in order to manipulate specific genetic traits of the fish. This work will have applications in several areas of marine biotechnology including aquaculture, production of natural products from marine organisms and basic studies of endocrine factors controlling fish growth and development.

Summary of Progress

During the course of this project methods were developed for the culture of PGCs derived from zebrafish embryos. Work was completed demonstrating that the cultured cells are able to produce viable germ cells when introduced into a host embryo and the cultures were used to introduce foreign DNA into the germ line resulting in the production of a transgenic line of zebrafish.

Accomplishments

The results from this research lay the foundation for the employment of a cell-mediated approach to gene transfer in fish. Once it is fully developed, this technology will provide an efficient method for altering specific genetic characteristics of fish in order to make a particular species more suitable for aquaculture production and provide a method to study the role of individual hormones and growth factors in controlling fish reproduction and growth.

Narrative Report

The goal of this project is to develop an efficient method for introducing foreign DNA into fish embryos to generate transgenic fish. The approach that we are developing relies on the use of cultured cells as a shuttle for introducing the foreign DNA into a fish embryo. This approach provides the advantage of enabling researchers to produce lines of fish that overexpress a particular foreign gene or produce fish that possess a targeted mutation that serves to inactivate a specific endogenous gene. Such an approach can be employed to manipulate genetic characteristics that are important for aquaculture production such as fertility, growth rate, temperature tolerance and disease resistance. The technology is also valuable for basic studies of gene function and hormone action during fish development and growth. To develop this technology, we are working to establish methods for the in vitro culture of fish primordial germ cells (PGCs). During embryonic development the PGCs are destined to become the eggs and sperm in the sexually mature fish. The ability to propagate PGCs in culture will enable researchers to genetically alter the cells by introducing foreign DNA into the cultures. Cells that possess the desired genetic alteration can then be selected in vitro and propagated. The cells may be selected for their ability to overexpress a foreign gene or alternatively, they may be selected for the presence of a specific mutation that inactivates an endogenous gene. When the selected cells are reintroduced into a developing host embryo by microinjection they will participate in embryonic development and contribute to the host germ line thereby transferring the genetic alteration to the eggs or sperm of the host as it becomes sexually mature. By breeding these chimeric individuals a transgenic line of fish will be established that possesses the desired genetic alteration.

During the course of this work we have developed methods for the in vitro culture of PGCs derived from zebrafish embryos. The PGCs were identified by the expression of the germ cell marker gene, *vasa*. Cells expressing *vasa* were identified by immunohistochemical staining with an antibody that recognizes the *vasa* protein, by reverse transcription polymerase chain reaction (RT-PCR) and by in situ hybridization using a *vasa*-specific probe. Using the methods that we developed, the PGCs were propagated for several weeks in culture. When introduced into a host embryo by microinjection the cultured PGCs were able to participate in development and produce viable germ cells in the host fish. Germ line contribution was demonstrated by breeding chimeric fish and identifying F1 individuals that possessed transgenic sequences and a pigmentation pattern that was donated by the cultured cells. To demonstrate the feasibility of using the PGC cultures for gene transfer into fish embryos, the cultures were employed to generate a transgenic line of fish carrying the bacterial marker gene, *neo*.

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- **Initiation Date**: March 1, 1998
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