

# **Zebrafish Embryology**

## ***An Introduction to Development and Differentiation***

### **Pre-visit Preparation**

Welcome to the University of Maryland Biotechnology Institute's (UMBI) Science and Technology Education Program. We hope that these materials are useful and will help prepare your students for a unique and exciting "hands-on" laboratory experience. We encourage you to review these pre-visit materials. The teacher background sheets are designed to increase your understanding of this topic. Students will have a richer experience with us if you go over the pre-visit materials with your class before your visit. For more information regarding The Science and Technology Education Program (SciTech), please visit our website: [www.umbi.umd.edu/~scitech](http://www.umbi.umd.edu/~scitech).

### **Summary of Student Experience**

After a guided inquiry about zebrafish, embryology and differentiation, students will investigate the effect of temperature on zebrafish embryos. Some students will investigate the effect of temperature on development, while others will investigate the effect of temperature on heart rate. Since students will be working with a living organism we ask that they treat it with respect and follow the appropriate guidelines defined by our staff on the day of the trip. Upon request, a Center of Marine Biotechnology (COMB) scientist will discuss his or her research, personal science career path and respond to student questions about possible careers in science.

### **Tips for a Successful SciTech Experience**

The Zebrafish Embryology Lab offers some unique opportunities for students and teachers. The Olympus America, Inc. microscope equipment is set-up so that you can videotape and photograph the many stages of zebrafish. We have a total of 30 stereomicroscopes and a video monitor that can display images of embryos under several of our microscopes, allowing students to view interesting findings that their classmates have discovered. Also, we have purchased a state of the art inverted microscope from Olympus that allows students to visualize highly magnified images (up to 400X) of the embryos. To take full advantage of this opportunity, please bring:

- A VHS videotape for recording,
- A roll of 35 mm print or slide film, 400 or 800 speed, and
- Some blank computer discs for digital pictures, Mac or PC format.

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# Experimental Design Background

## Stating the Question

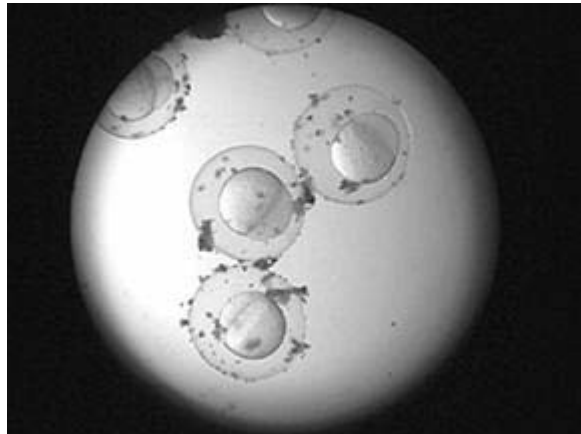
Every experiment begins with a question that the experiment will be designed to answer. Formulating this question is often the most difficult and most essential part of setting up a research project. There are many questions that could be investigated during the exploration of zebrafish development. We will be focusing on the question, “What is the effect of temperature on embryo development?”

## Hypothesis

The clearest way to write a hypothesis is to use “if...then” statements. For example: “If a zebrafish embryo is placed in a colder temperature, then development will be slower than at a normal temperature.” The most common hypothesis is the null hypothesis, which simply states that the variable or experimental situation being tested will exhibit no significant difference from the controls. For example, one null hypothesis could state, “There will be no difference in the development of embryos at different temperatures.”

## Controls

When designing an experiment, it is important to plan ahead so that the method you are testing is compared against a standard. In this experiment, we will be examining the development and the heart rate of zebrafish embryos at three different temperatures. These temperatures are 5 °C , 20 °C, and 28 °C. The normal, or standard, temperature for zebrafish is 28 °C.



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## Zebrafish Background

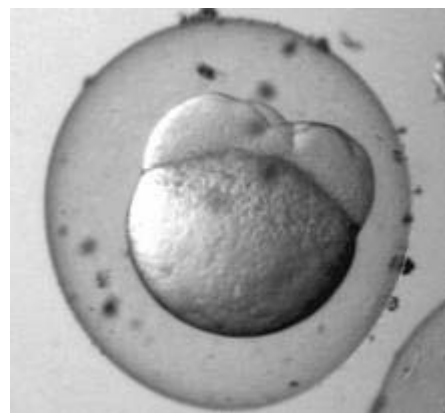
### Classified Information

Zebrafish (*Danio rerio*) are freshwater tropical fish originating from the Coromandel Coast of India. Zebrafish live in small, clear, swift running streams. Besides being used in scientific research, zebrafish are common, inexpensive aquarium fish that are sold in pet stores. A number of biologists and geneticists have set aside the traditional animal models (e.g. frogs, fruit flies, chickens, worms and mice) for the more versatile zebrafish. There are many advantages to using zebrafish as a model for human development. First, the zebrafish is more closely related to humans than many of the other animal models. Though mice are more similar to humans than zebrafish, mouse embryo development takes place inside the mother, while zebrafish embryos develop outside the mother, enabling the scientists to see the developing egg. Since zebrafish lay eggs, it is easier to perform artificial insemination and transgenetics in the zebrafish than in the mouse. Another advantage to zebrafish is that it is easy to collect a large number of eggs from relatively few fish. The eggs are clear and are easily seen under a dissecting microscope. In addition, the eggs develop quickly and hatch in three to four days, an advantage to scientists because results can be obtained in a short amount of time.

### Developmental Biology

People have always been fascinated by the early development of animals. They have been wondering about the mystery of life's earliest beginnings for a very long time. Aristotle wrote the earliest known animal biology text, *Historia Animalium*, and later a companion text focusing on developmental biology, *De Generatione Animalium*.

We have come to understand that, from worms to humans, development is remarkably similar. One of the great questions in developmental biology has been how embryonic cells activate and inactivate genes to form tissues. The discovery of these mechanisms in fruit flies *Drosophila melanogaster* resulted in the awarding of the Nobel Prize to three developmental biologists, Eric Weischaus, Christaine Nusslein-Volharda and Edward B. Lewis. In a developing fertilized egg, a set of genes called homeobox genes determines which end becomes the head. Homeoboxes are fascinating because they occur in a wide variety of organisms, including fish, mice, fruit flies, and humans. From fly to man, cells obey similar signals.

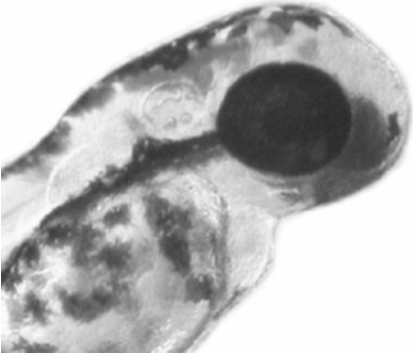


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The universality of signaling gives researchers a handy tool, the ability to use one easily grown species to study another species that is more difficult to manage in a laboratory.



For example, striped bass populations have made a remarkable recovery in the Chesapeake Bay because of the stringent conservation plan that was implemented nearly ten years ago. However, the demand for these fish still exceeds the supply. Sport and commercial fishermen prize this tasty fish. Scientists at the Center of Marine Biotechnology (COMB) are working to improve aquaculture techniques in order to increase the supply of striped bass. Unfortunately many wild species of fish, including striped bass, are difficult to breed. The

problem has been tentatively traced to a small number of hormones within the brain. Apparently, the gene that controls reproductive hormones is switched off in captivity. Researchers at COMB are looking for the gene responsible for this switch. Using zebrafish as a model system enables researchers to more easily study ways of inducing striped bass to reproduce in aquaculture.

## Genetic Analysis

A gene is a locus on a chromosome that encodes a specific protein or several related proteins. It is considered the functional unit of heredity. Genes direct the synthesis of proteins. A mutation in a gene can result in a nonfunctional protein. This defective protein could result in the disruption of an essential biological process. In order to understand the function of essential proteins, scientists alter genes to create mutations that result in the production of nonfunctional proteins. Much can be learned about the function of a normal protein by understanding what is wrong with a defective protein. When scientists discover a mutation in a zebrafish that causes a protein to not function, they can search the database of the human genome to search for a similar gene in humans. Scientists can also study the development of the zebrafish embryo to help discover the causes of birth defects in humans. By understanding how and when different genes are turned on to cause cells to differentiate into different parts of the body, scientists hope to prevent these birth defects.

## Recent Zebrafish Research

Recent zebrafish research focuses on how to control the hormonal processes that stimulate breeding. The zebrafish could provide scientists with a living model for the study of these same processes in other fish, such as the striped bass. If scientists can isolate the genes that make aquaculture of striped bass difficult and transfer them to zebrafish, then the easily grown zebrafish can be used as a model to study those genes. The transfer of genes from the striped bass to the zebrafish would make the

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zebrafish a transgenic animal. Transgenic animals and plants are widely used in genetic research in science labs around the world.

Other zebrafish studies involve using them as a model for human development. Dr. Leonard Zon of Massachusetts General Hospital has discovered a zebrafish gene that controls iron transport and matches a gene on the human gene sequence map. Another researcher at Massachusetts General Hospital, Mark Fishman, studies defects in heart development and his group has found a gene that directs the formation of blood vessels in zebrafish. Didier Stainier and Debbie Yelon also study heart development in zebrafish. Didier Stainier of the University California – San Francisco identified a gene for a protein that tells cardiac stem cells how to form a beating heart.



Here at University of Maryland Biotechnology Institute's Center of Marine Biotechnology, Dr. Jim Du studies how a fertilized egg can develop into an animal with thousands of distinct cell types. He and his co-workers use zebrafish as a model system to investigate the formation and differentiation of vertebrate muscle cells during embryogenesis. He has found that a growth factor, the Hedgehog protein, plays an important role in the development of slow muscle cells. By transferring an extra

Hedgehog protein gene into the embryo, Dr. Du observed that more slow muscle cells were formed, and the excess of the protein blocked the formation of fast muscle cells. This indicates that the Hedgehog protein acts as a positive signal to specify slow muscle cells. Dr. Du is currently looking for the signal involved in the formation of fast muscle cells.

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## Glossary

**Cell** The fundamental structural unit of living things. A cell could be a singular living organism or a component of tissue in multicellular organisms.

**Chromosome** A linear or circular strand of DNA (and associated proteins) that contains multiple genes.

**Development** The act of progressing from fertilized egg to later stages of maturation.

**Differentiation** Process by which cells change to a more specialized form or function during development.

**Embryo** A term which defines a number of developmental stages of a fertilized egg as it progresses from a few cells to a fully developed organism.

**Embryology** The study of the developmental stages of an organism starting with the fertilized egg.

**Gene** A sequence of DNA that controls transmission and expression of one or more traits by specifying the structure of a protein. The functional unit of inheritance.

**Transgenic** An organism that has a foreign gene or genes introduced into their genetic material.

**Zebrafish** A common brackish water tropical fish which has a prolific breeding cycle.

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## Book & Magazine References

Nusslein-Volhard, Christiane. "Gradients That Organize Embryo Development", *Scientific American*, August 1996. pp. 54-61.

*From Egg to Adult*. Howard Hughes Medical Institute Report, May 1992.

Westerfield, Monte. *The Zebrafish Book*. Institute of Neuroscience, University of Oregon Press, Eugene, OR. 1995.

Sobel, R.K.2000. Secrets of biology's new supermodels. U.S. News & World Report, December 11. p.66.

Sprague, J. Doerry, E., Douglas, S. and Westerfield, M. 2001. The Zebrafish Information Network (ZFIN): a resource for genetic, genomic and developmental research. *Nucleic Acids Research* 29:87-90. World Wide Web URL: <http://zfin.org/>

## Web References

<http://depts.washington.edu/fishscop/>

[http://worms.zoology.wisc.edu/embryology\\_main.html](http://worms.zoology.wisc.edu/embryology_main.html)

[http://ucalgary.ca/UofC/eduweb/virtualembryo/why\\_fish.html](http://ucalgary.ca/UofC/eduweb/virtualembryo/why_fish.html)

<http://zfin.org/>

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## Maryland State Department of Education Core Learning Goals

The following Core Learning Goals link directly to the UMBI Science and Technology Education Program's Zebrafish Embryology Lab. Take a few moments to review the specific goals, expectations, and indicators below so that you may prepare your students appropriately. The parts of our program that integrate the indicators are found in parentheses below. If you do not have a Core Learning Goals document or CD talk to your department chair or contact your science supervisor.

### Core Learning Goal – Science

#### Goal 1 - Skills and Processes

**Expectation 1.2** - The student will pose scientific questions and suggest investigative approaches to provide answers to questions.

*Indicator 1.2.1* -

- The student will identify meaningful, answerable scientific questions.

*Indicator 1.2.2* -

- The student will pose meaningful, answerable scientific questions.

*Indicator 1.2.3* -

- The student will formulate a working hypothesis.

*Indicator 1.2.4* –

- The student will test a working hypothesis.

*Indicator 1.2.5* -

- The student will select appropriate instruments and materials to conduct an investigation.

**Expectation 1.3** - The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.

*Indicator 1.3.4* -

- The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.

**Expectation 1.4** - The student will demonstrate that data analysis is a vital aspect of the processes of scientific inquiry and communication.

*Indicator 1.4.9* -

- The student will use analyzed data to confirm, modify, or reject an hypothesis.

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**Expectation 1.5** - The student will use the appropriate methods for communicating in writing and orally the processes and results of scientific investigation.

*Indicator 1.5.1 –*

- The student will demonstrate the ability to summarize data (measurements/observations).

*Indicator 1.5.2 -*

- The student will explain scientific concepts and processes through drawing, writing, and/or oral communication.

**Expectation 1.7** - The student will show that connections exist both within the various fields of science and among science and other disciplines including mathematics, social studies, language arts, fine arts, and technology.

*Indicator 1.7.5 -*

- The student will investigate career possibilities in the various areas of science.

### **Goal 3 - Concepts of Biology**

**Expectation 3.2** - The student will demonstrate an understanding that all organisms are composed of cells which can function independently or as part of multi-cellular organisms.

*Indicator 3.2.2 -*

- The student will conclude that cells exist within a narrow range of environmental conditions and changes to that environment, either naturally occurring or induced, may cause changes in the metabolic activity of the cell or organism.

**Expectation 3.5** - The student will investigate the interdependence of diverse living organisms and their interactions with the components of the biosphere.

*Indicator 3.5.3 -*

- The student will investigate how natural and man-made changes in environmental conditions will affect individual organisms and the dynamics of populations.

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