Heat shock of Zebrafish (*Danio rerio*) embryo causes disruptions in somite development

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Objective

Heat shock treatments have been shown to produce disturbances in zebrafish (*Danio rerio*) somite development (Roy et al., 1999). Embryos will be exposed to a brief heat shock to induce defects in the formation of a repeating structure.

Introduction

Segmentation during development is a common phenomenon in vertebrates. It involves the anterior/posterior body axis dividing into repeating segments that provides a body plan (Holley and Takeda, 2002). Structures such as rhombomeres of the hindbrain, branchial arches, and trunk somites are created through segmentation (Roy et al., 1999).

Somites in vertebrates are formed most commonly through the epithelial-mesenchymal transition of the cells lying within the segmental plate mesoderm. Beginning at the conclusion of gastrulation of zebrafish, one pair of somites forms from the presomitic mesoderm by migration of cells within the extending tailbud every 30 minutes until there are 30 pairs (Holley and Takeda, 2002). The *hairy* gene, expressed in a dynamic pattern during somitogenesis, correlates with the positioning of where a somite will separate from the unsegmented mesoderm (Gilbert, 2010). However, it is still somewhat unclear how a series of similar, repeated units differentiate to produce individually fated structures in a precisely coordinated fashion.

Recent study suggests that there is an oscillator mechanism that mediates repeated cycles of gene expression and repression of the *Notch* gene (Holley and Takeda, 2002). In *Notch* and Notch pathway mutations, the proper segmentation of somites fails, and somite boundaries are absent or irregular (Jiang et al., 2000). Forsberg and colleagues have found in mice that *Lunatic fringe* expression precedes somite boundary formation (1998). Indeed, *Fringe* genes regulate the Notch signaling pathway, and *L-fringe* is expressed as a dynamic and repetitive wave that takes four hours to complete one cycle. Termination of the wave occurs immediately prior to somite boundary formation.

It has been shown that heat shock treatments affect the presmoitic mesoderm. There is a zone in which there is normal somite development before a defective somite appears (Roy et al., 1999). After the defect, normal development resumes. Thus, it appears to affect a particular stage during the oscillation. In conjunction with research in the Notch pathway, investigation into the effects of heat shock will provide insights into the mechanism of somite formation.

Methods

Fish care

Adult zebrafish were maintained at 28.5°C on a 14h light/10h dark cycle. Embryos were collected from natural spawnings and maintained in embryo medium (NaH2PO4 0.01M 10mL; Na2HPO4 0.01M 10mL; Sodium citrate 0.1M 20mL; CaCl2 0.1M 15mL; dH20 945 mL) at 28.5°C.

Treatment

Embryos were transferred into test medium using wide-bore pasture pipettes. Embryos were grouped by stage as observed through a light microscope and allowed to develop at 28.5°C. After reaching somite stage, approximately 10-11 hours after fertilization, embryos were removed and placed in glass test tubes containing embryo medium. The test tubes were then placed in a 40.0°C water bath and embryos were heat shocked for 30 minutes. After this period, the embryos were returned to medium at 28.5°C and incubated for 24 hours.

Visualization

Observe after 24 hours using a dissecting microscope. Embryos were.

To visualize motoneuron axons, embryos were dechorionated using fine forceps, fixed in 4% paraformaldehyde (PFA) overnight, and stained with antibody according to methods detailed in The Zebrafish Book (Westerfield, 1993).

Embryos were mounted on depression slides for observation under a microscope and photographed using an Olympus DP12 camera.

Prep List

Warm water incubator set at 40.0°C
Incubator set at 28.5°C
Wide-bore pasture pipettes
Fine forceps
Glass Pyrex dishes for collection and incubation of embryos
Zebrafish embryo medium
Dissecting microscope

Literature Cited

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Fish/fish_solutions.html
http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Student/Heatshock/heatshock.html

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