Retinoic acid-induced truncation of zebrafish (Danio rerio) embryos

Sean Anderson (Swarthmore College), Adam Ligas (F&M College) and
Judith Cebra-Thomas (Millersville University)

Objective

The purpose of this experiment is to examine the effects of treating zebrafish (*Danio rerio*) embryos with varying concentrations of retinoic acid

Introduction

Retinoic acid (RA) is a highly teratogenic derivative of vitamin A that is known to influence *Hox* gene expression. RA-mediated gene activation is important for normal vertebrate development; RA acts as a posteriorizing signal in many developing systems, including mammals, and is also involved in limb formation (Gilbert, 2003).

When embryonic exposure is higher than normal, however, developmental anomalies occur. Exposure of the human fetus to 13-cis-retinoic acid results in a characteristic pattern of anomalies, including absent or defective ears, absent or small jaws, cleft palate, aortic arch abnormalities, thymic deficiencies, and abnormalities of the central nervous system. Similar anomalies are observed in other vertebrates. In mice, for example, embryonic exposure to retinoic acid results in axial truncation and causes a dramatic reduction in the sizes of the first and second pharyngeal arches, which normally form the jaw, ear, and other facial bones (Gilbert, 2003). The truncated embryo exhibits a posterior region having the characteristics of the anterior region of an embryo that had developed normally, including a posterior extension of the ribcage. At very high concentrations, the cells do not differentiate to form the posterior of the embryo at all (Modak et al., 1993).

Retinoic acid disrupts development by altering the expression of *Hox* genes, causing the re-specification of the anterior-posterior axis and inhibition of neural crest cell migration from the cranial region of the neural tube (Gilbert, 2003). Retinoic acid binds to specific retinoic acid receptors (RAR; Hyatt et al. 1992). After binding, the receptor becomes an active transcription factor. The retinoic acid-bound RARs have at least two modes of action, one of which is to bind to their DNA enhancer sequences and activate particular genes that are not usually activated in these cells. These genes include certain homeotic genes that specify the anterior-posterior position along the body axis. In this way, they can cause homeotic transformations, generally converting anterior structures into more posterior structures (Gilbert, 2003).

Zebrafish embryos treated withy retinoic acid have been shown to exhibit truncation similar to that observed in mammalian embryos (Holder & Hill, 1991). In order to further examine the effect of retinoic acid on zebrafish embryos and to determine whether the magnitude of such effects is concentration dependent, the embryos will be treated with different concentrations of retinoic acid and allowed to continue development.

Materials

Mature zebrafish
Zebrafish Embryo Medium (ZFEM)
all-*trans*-retinoic acid (Sigma R2625), stock concentration 10^{-4} M in DMSO
DMSO

60 mm glass Petri dishes
wide-mouth glass Pastuer pipets
Siphon and fine fry net or mesh filter
Incubator (28°C)
Dissecting microscope and camera

Procedure

1.Obtain dome/ 30% epiboly stage (4-5 hours post-fertilization at room temperature) zebrafish embryos

2. Dilute 10^{-4} M retinoic acid stock using ZFEM:

10^{-8} M (1.0 μL stock solution in 10 mL ZFEM)

10^{-9} M (1mL 10^{-8} M solution in 10 mL ZFEM)

Place approximately 5 mL of each titration into an appropriately labeled Petri dish. For the control, use 5 mL of ZFEM.

3. Transfer 10 -15 zebrafish embryos into each Petri dish.

4. Incubate embryos at 28°C overnight.

5. Observe embryos for malformations using the dissecting microscope. The embryos may need to be dechorionated, which may be done under the dissecting microscope with clean fine forceps by gently tearing the chorion and allowing the embryo to pass though.

References

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