

Ultraviolet Irradiation Impairs Zebrafish Epiboly

Zena Zellers and Judith Cebra-Thomas

Millersville University

Objective: The objective of this experiment was to treat zebrafish embryos with UV light to investigate the effect of UV exposure on epiboly and other embryonic processes.

Introduction

Zebrafish lay transparent eggs which develop quickly around an undivided yolk cell. First, the cells develop on one side of the yolk, but they eventually undergo epiboly, where they spread out and surround the yolk cell. From there, somites, eyes, ears, a brain, and other organs begin to form a complete embryo, until the zebrafish is completely formed and ready to hatch (Kimmel et al, 2005). It has been proposed that epiboly is powered by microtubules in the developing embryo, and that ultraviolet irradiation affects these microtubules (Strahle and Jesuthasan, 1993). Therefore, epiboly will be impeded more with increasing ultraviolet irradiation and the embryos will be less likely to fully develop with a higher UV exposure. This is important because there is concern for many animals due to the rising levels of ultraviolet exposure on Earth due to holes in the ozone layer of our atmosphere, which can cause different defects in embryos of many species.

Materials

Mature zebrafish

Zebrafish Embryo Medium

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

Handheld UV light units

Procedure

1. To collect the embryos, put a layer of marbles on the bottom of the zebrafish tank, and allowed about an hour after their day began for the fish to lay their eggs.
2. Collect the embryos with a vacuum filtration system, and sorted the embryos into four groups of fifteen embryos. Select embryos in the late cleavage phase (e.g. the 64-cell stage, Figure 1), and ensure that all embryos are healthy.
3. Transfer groups of 10-20 embryos to 60 mm glass Petri dish in embryo media. The first petri dish of embryos is the control group, which was not exposed to any UV light.
4. Expose the subsequent dishes varying amount of UV light. Each light source is different; each group should try several exposure times (1, 2 & 5 minutes for example) with the light source a fixed height (record!) above the embryos in uncovered petri dishes. Shorter wavelengths (e.g. 254 nm) will have a stronger effect on development, but longer wavelengths (e.g. 366 nm) are more physiologically relevant.
5. Incubate the embryos at 28 degrees Celsius in tank water for roughly 24 hours. Record any abnormalities that you see in your samples.



Figure 1: An embryo in the 64-cell stage.

References

- Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F. 1995. Stages of Embryonic Development of the Zebrafish. *Developmental Dynamics* **203**: 253-310.
- Strahle, U., and S. Jesithasan. 1993. Ultraviolet Irradiation Impairs Epiboly in Zebrafish Embryos: Evidence for Microtubule-dependent Mechanism of Epiboly." *Development* **119**: 909-19.