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Expression of Bik, a Pro-Apoptotic Protein, in Developing Zebrafish (Danio rerio) Oocytes

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Expression of Bik, a Pro-Apoptotic Protein, in Developing Zebrafish (*Danio rerio*) Oocytes

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Abstract

I addressed the role of cell death (apoptosis) within the process of egg cell development in zebrafish, *in vitro*. Current data on the role of apoptosis in oocyte maturation of zebrafish are conflicting, and little is known about apoptosis regulation at the individual stages of oocyte development.

The importance of apoptosis will be determined by recording the relative expression of Bik, a key protein involved in the apoptosis pathway, between zebrafish oocytes that have been induced to mature and those that are left alone. Relative expression of Bik will be measured by Western blot. I expect that Bik expression, and rates of apoptosis, will be relatively higher in cells that will undergo maturation. Meiotic checkpoints in meiosis will provide a means of selection as the cells mature.

Additionally, maturing and non-maturing oocytes will be exposed to nocodazole, an apoptosis-inducing drug, as a positive control. We found that regardless of treatment, oocytes underwent apoptosis, though hCG may have hastened the process.

Introduction

Conflicting data have been presented on the role of apoptosis in teleost (the subfamily zebrafish belong to) oocyte development. This information is valuable if only because of zebrafish's role as a model organism for vertebrate development.

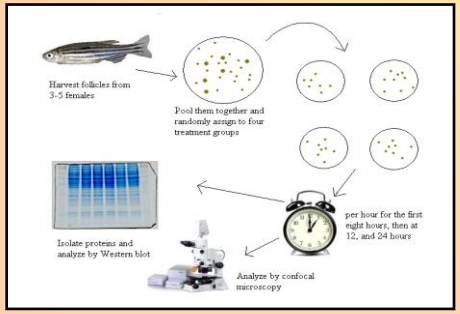
Objectives

1. Correlate Bik expression with observed visual cues for apoptosis as part of naturally occurring cell death.
2. Show that Bik is upregulated as part of apoptosis in response to induced cell disruption.

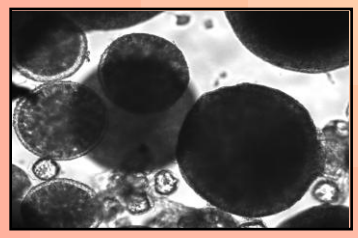
Materials and Methods

I collected and pooled developing oocytes from a number of zebrafish and incubated them with human chorionic gonadotropin (hCG) or nocodazole. I removed portions of oocytes at set points throughout the incubation (1, 2, 4, 6, 10, 12, and 24 hours). The oocytes were fixed for TUNEL assay or analyzed by Western Blot.

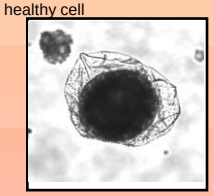
The TUNEL assay provided a qualitative way in which to observe cells undergoing apoptosis. Analysis by Western blot would allow for a quantitative measurement of apoptosis occuring in the cells.

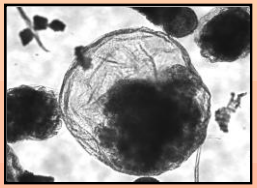


Normal, healthy cells vs. apoptotic cells



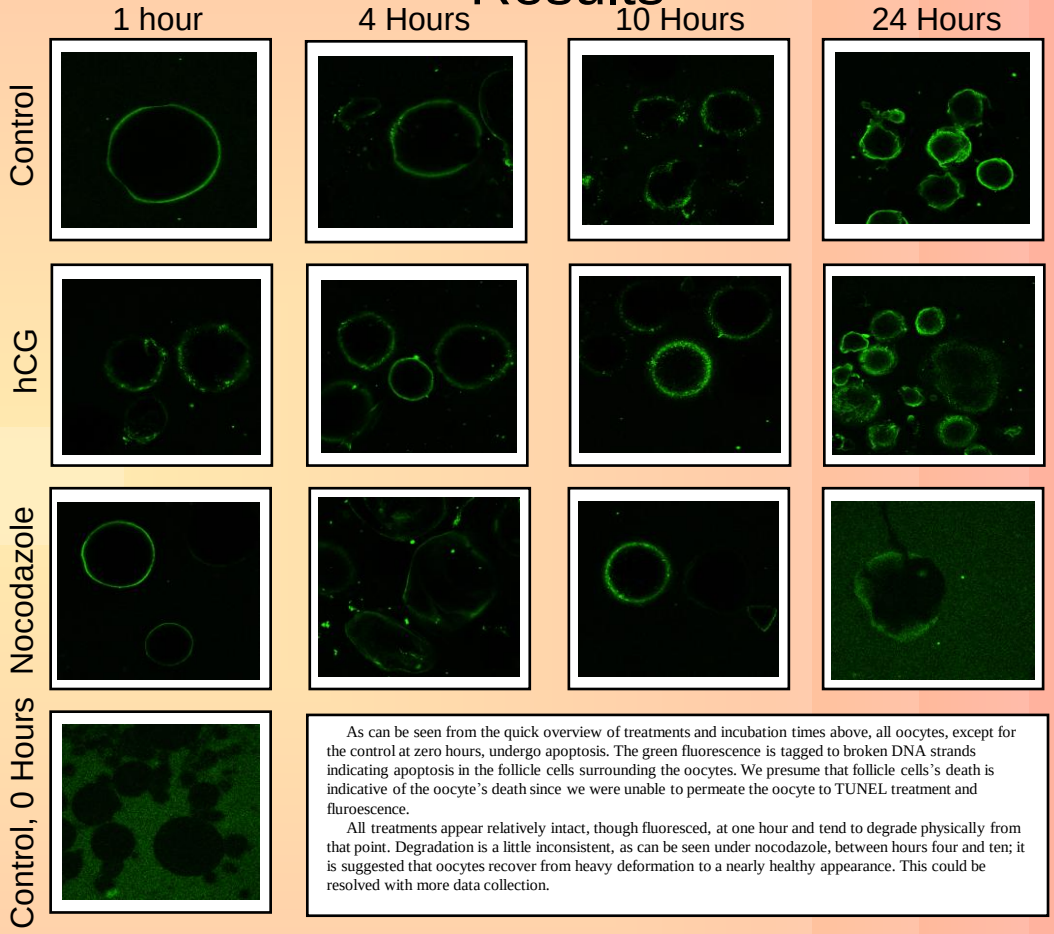
←round cells with dense yolk proteins at 6 hours indicate a healthy cell





Apoptotic cells typically appear wrinkled and deformed as they degrade

Results



As can be seen from the quick overview of treatments and incubation times above, all oocytes, except for the control at zero hours, undergo apoptosis. The green fluorescence is tagged to broken DNA strands indicating apoptosis in the follicle cells surrounding the oocytes. We presume that follicle cells's death is indicative of the oocyte's death since we were unable to permeate the oocyte to TUNEL treatment and fluroescence.

All treatments appear relatively intact, though fluoresced, at one hour and tend to degrade physically from that point. Degradation is a little inconsistent, as can be seen under nocodazole, between hours four and ten; it is suggested that oocytes recover from heavy deformation to a nearly healthy appearance. This could be resolved with more data collection.

Conclusions and Future Directions

The data collected were insufficient to draw any reliable conclusions about the role of apoptosis in zebrafish oocyte development. We were not able to identify any overlying trends that might indicate whether artificial maturation *in vitro* enacted apoptosis within the developing oocytes. However, it is apparent that apoptosis is occuring throughout incubation and, overall, cells' conditions seem to worsen as time passes, regardless of treatment. With our limited data, it is difficult to tell whether this significant or not.

In this study, we experienced much difficulty creating Western blots that yielded the necessary information. For future research, I'd recommend looking at RNA expression through rtPCR or fine tuning the Western blot procedure before data collection. Additionally, it would be beneficial to stain the TUNEL assays with DAPI as well as the normal fluorescence, in order to see all cells, not just those undergoing apoptosis. In future studies, it would be interesting to examine the relative efficiency of a number of maturation-inducing hormones and steroids, and how they might interact together to affect oocyte maturation.

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