

Summer 2014

24 hour incubation of ovary tissue in $17\alpha 20\beta$ - Dihydroxy-4-pregnon-3-one causes Bcl-2 and Pi3K- III expression to vary in *Danio rerio*

Beth Onaga
bonaga@pugetsound.edu

Follow this and additional works at: http://soundideas.pugetsound.edu/summer_research

 Part of the [Cell Biology Commons](#), [Developmental Biology Commons](#), and the [Molecular Biology Commons](#)

Recommended Citation

Onaga, Beth, "24 hour incubation of ovary tissue in $17\alpha 20\beta$ -Dihydroxy-4-pregnon-3-one causes Bcl-2 and Pi3K-III expression to vary in *Danio rerio*" (2014). *Summer Research*. Paper 223.
http://soundideas.pugetsound.edu/summer_research/223

This Article is brought to you for free and open access by Sound Ideas. It has been accepted for inclusion in Summer Research by an authorized administrator of Sound Ideas. For more information, please contact soundideas@pugetsound.edu.

24 hour incubation of ovary tissue in 17 α 20 β -Dihydroxy-4-pregnon-3-one (DHP) causes Bcl-2 and Pi3K-III expression to vary in *Danio rerio*

Beth Onaga* and Alyce DeMarais

Biology Department, University of Puget Sound 1500 N. Warner Ave Tacoma WA 98416

*bonaga@pugetsound.edu

Abstract

The present study investigated the effects of increasing concentrations of progesterone on cell death by monitoring the expression of mRNAs (Bcl-2 and Pi3K-III) specific for apoptosis and autophagy in *Danio rerio* ovary tissue using quantitative (q)PCR. Progesterone is used to regulate oocyte maturation *in vivo* (Clelland, 2009); however, synthetic progesterone in oral contraceptives is found in the environment. Primers for Bcl-2 and Pi3K-III were used to investigate gene activity via mRNA produced. The results of the present study reveal that gene activity was variable as revealed by qPCR analysis. After 24 hour exposure to progesterone Bcl-2 and Pi3K-III expression was inconsistent.

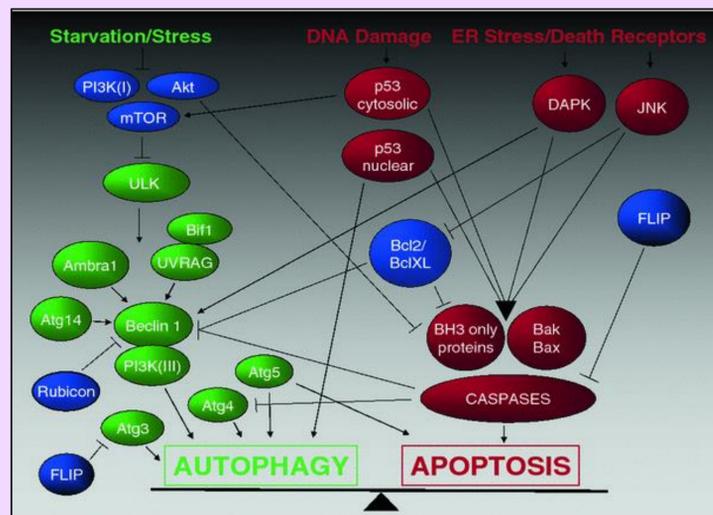


Figure 1. Interplay between autophagy and apoptosis (Fimia, 2010). Apoptosis is programmed cell death activated by the caspases whereas autophagy is a type of cell death that utilizes autophagosomes to digest organelles or whole cells. Bcl-2 acts as both an apoptotic and autophagic inhibitor. Autophagy can be activated by the formation of the Beclin 1-Pi3K-III complex.

Objectives

- Design and optimize primers for the Bcl-2 and Pi3K-III for use in quantitative (q)PCR technology.
- Investigate how various concentrations of progesterone affect egg development and follicle degradation.

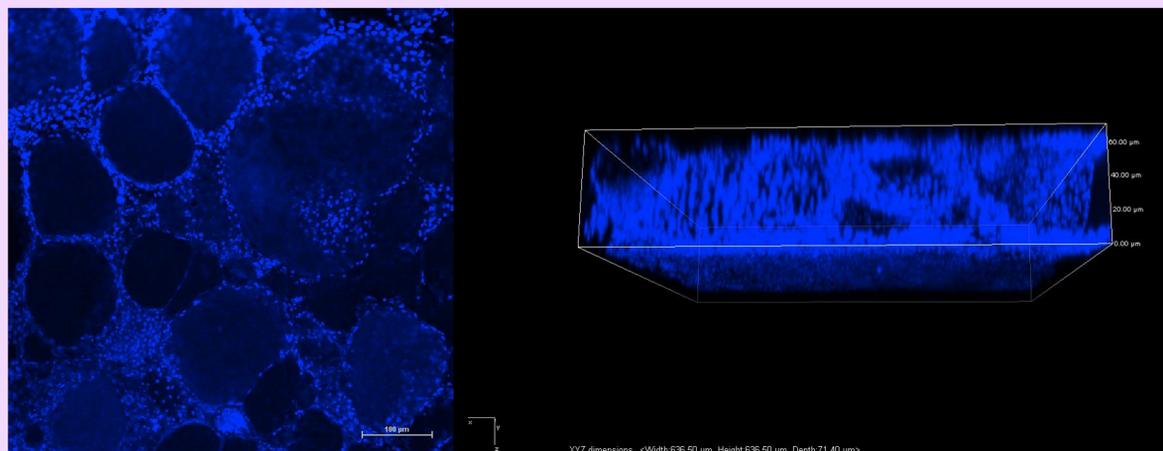


Figure 2. Untreated ovary tissue with DAPI staining using confocal microscopy. The follicle cells are visible in the top of the sample (left) where they surround the developing oocyte. The ovary contains a complex community of various cells (right) including stroma, oocytes, and various types of follicle cells.

Acknowledgements

Thank you to the University of Puget Sound for the General Award and UEC funding. Thank you especially to Amy Replogle for helping our lab with primer design as well as confocal microscopy usage. Lastly thank you to everyone in the DeMarais lab.

Materials and Methods

Primer Optimization

- Desired gene sequences were acquired from the National Center for Biotechnology Information and Primer3 tools from the University of Massachusetts Medical School.
- Primers were optimized using the SYBR green method in an iQ5 iCycler thermal cycler (Bio-Rad).

Experimental Set-up

- Ovary tissue was isolated from three zebrafish and incubated at 26°C for 24 hr in L-15 medium (with 1x antibiotic/ antimycotic) with synthetic progesterone (17 α 20 β DHP) (0, 1, 10, 100 ng/mL) for *in vitro* maturation.
- RNA was collected from tissue and converted to cDNA (using BioRad kits) then stored at -20°C.
- qPCR was used to monitor and measure gene activity via mRNA produced.
- The amount of Bcl-2 and Pi3K-III activity was normalized to β -actin, a reference gene.

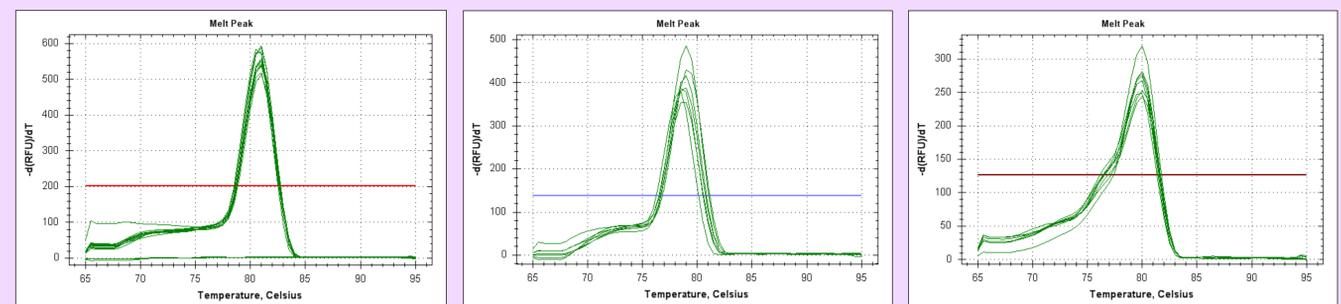


Figure 3. Establishing a temperature gradient for primer optimization. The melt curve for the reference gene β -actin (left) created a single peak thus successfully synthesizing a specific product. The Bcl-2 primer (center) contains multiple peaks indicating that it was not specific and possibly synthesized other products. Pi3K-III (right) contained a single peak for specific product. Optimal conditions for all primers were 61.5°C at 250nm concentrations for forward and reverse.

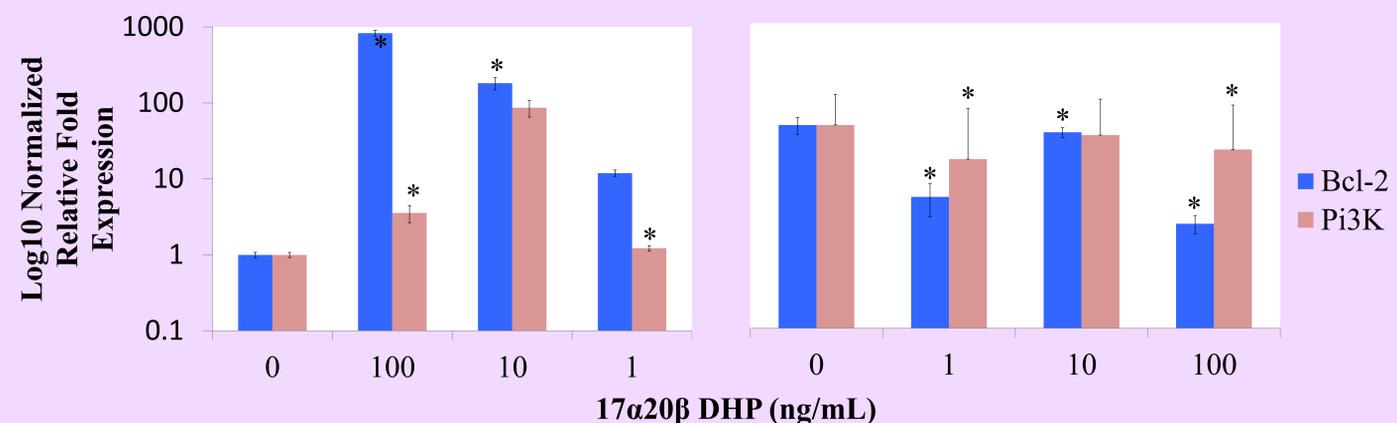


Figure 4. Monitoring apoptotic and autophagic activity after 24 hour incubation in DHP using qPCR technology. The first experiment (left) utilized ovary tissue from 3 different fish. The experiment was replicated (right) and the relative gene activity from both genes were inconsistent. Bcl-2 and Pi3K-III activity were normalized against β -actin. Data are the \pm mean s.d. *P<0.05 from the reference gene β -actin.

Future Directions

- Inconsistency in results may have been due to the various cell types present (Figure 2). For future experiments it may be beneficial to separate the eggs from follicle cells.
- Bcl-2 may not have synthesized a specific product, as implied by Figure 3, thus redesigning primers is necessary. If the primer is nonspecific it could yield inaccurate results via qPCR analysis.
- Redesign the reference gene primers. β -actin fluoresced sooner than Bcl-2 and Pi3K-III making comparisons inaccurate.

Literature Cited

Clelland E, Peng C: Endocrine/paracrine control of zebrafish ovarian development. (2009) *Molecular and Cellular Endocrinology*, **312**, 42-52
Fimia GM, Piacentini M: Regulation of autophagy in mammals and its interplay with apoptosis. (2010) *Cell. Mol. Life Sci.*, **67**, 1581-1588