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Determining the Role of Wnt Signaling in Zebrafish Oocyte Maturation Through Examination of β-catenin and Dishevelled mRNA Concentrations

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Determining the Role of Wnt Signaling in Zebrafish Oocyte Maturation Through Examination of β-catenin and Dishevelled mRNA Concentrations

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Introduction

During oocyte maturation, the oocyte progresses from prophase I to metaphase II of meiosis, and a multitude of other cellular changes occur[1]. Wnt singaling pathways are known to regulate gene expression, cell behavior, cell adhesion, and cell polarity, as well as play an essential role in embryonic development[2]. Because of this, I am examining the role of Wnt signaling pathways in the earlier process of oocyte maturation, specifically by looking at two Wnt signaling pathway components: β-catenin (*ctnnb1*) and Dishevelled (*dvl2*). β-catenin is a an interesting protein to study because it plays a dual role as both a cell adhesion protein when attached to membrane-bound complexes, and a coactivator for transcription by the Wnt pathway when free in the cytoplasm[2, 3]. Dishevelled is the "hub" of Wnt signaling and plays a key role in relaying external signals to internal pathway components[4]. Preliminary research has suggested that β-catenin increases in relative cytoplasmic concentration after maturation, and my findings from last summer showed that this change is not the result of migration from cytoskeleton associated membrane-bound complexes[5]. The first step in my research is to determine a reference gene for zebrafish oocyte maturation, as none are well classified for this specific scenario. I examined β –actin, GAPDH and ef1-α, as these were found to have constant expression during zebrafish embryo development or bovine oocyte development[6, 7]. This will be followed by examining the changes in mRNA concentrations for β-catenin and Dishevelled over the course of oocyte maturation in order to determine the role and importance of the Wnt signaling pathway in this process. Changes in mRNA concentrations are determined through real-time RTPCR analysis. The results of my research will contribute to our understanding of the cellular processes which occur during oocyte maturation, and the importance of signaling pathways such as the Wnt pathway in these processes.

Materials and Methods

- Primer design and optimization
- Redefinition of oocyte collection procedure
- Induction of oocyte maturation with 1 µg/mL progesterone (DHP)
- Incubation of oocytes for 0, 1, 2, 4, 8, and 24 hours
- RNA extraction with Qiagen RNAeasy Mini Kit and conversion to cDNA
- Testing possible reference genes through qPCR
  - *actb1*, *gapdh*, and *ef1a*
  - Check for constant expression throughout all time-points
- Measuring expression of target genes *ctnb1* and *dvl2* with qPCR
  - Run target gene against reference gene at all time-points.
  - Determine gene expression relative to expression of the reference gene

References

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5. Hamilton K: Possible role of β catenin in oocyte maturation in zebrafish. (2006) unpublished manuscript.
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Results

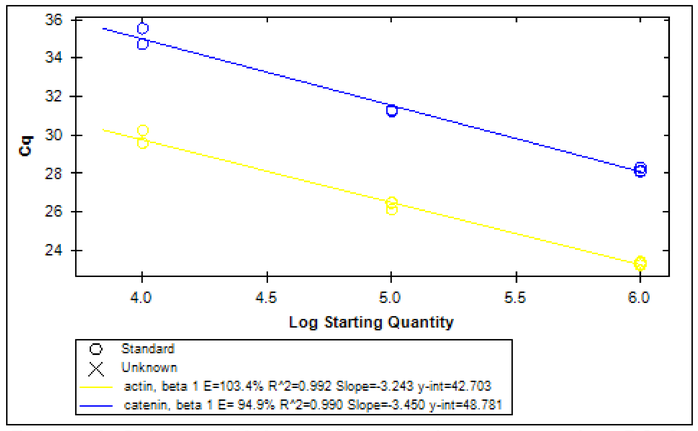

Figure 1. Primer efficiency for *actb1* and *ctnnb1* at 56°C

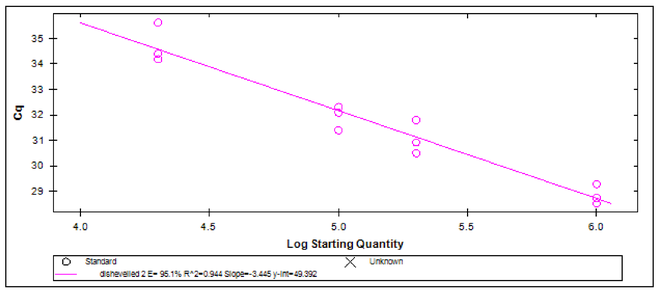

Figure 2. Primer efficiency for *dvl2* at 56°C

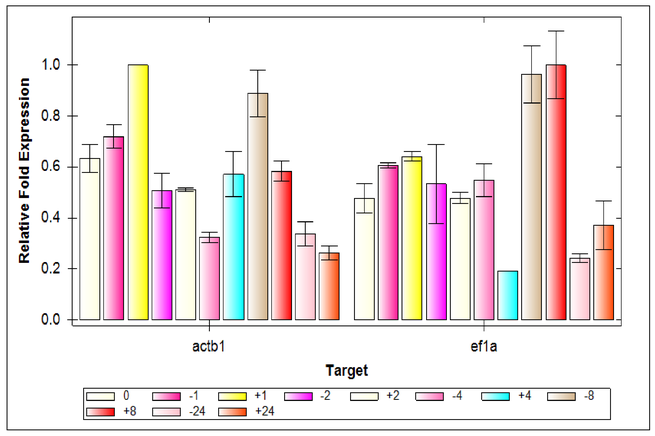

Figure 4. Gene expression for *actb1* and *ef1a* during maturation

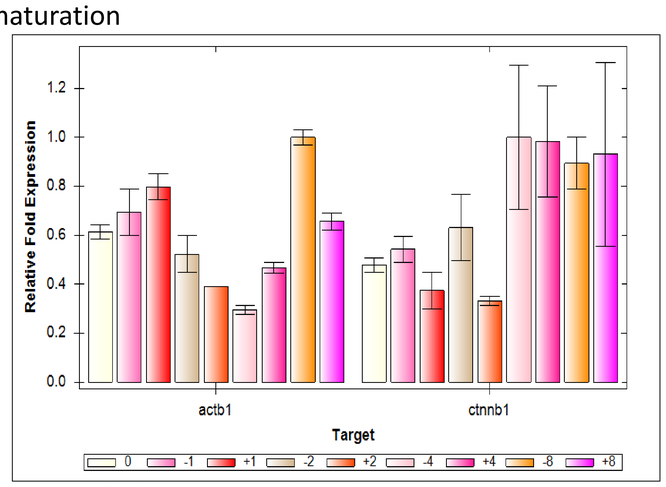

Figure 5. Gene expression for *actb1* and *ctnnb1* during maturation

Discussion

- All primers were successfully optimized for use in qPCR.
- Oocyte collection methods were redefined to include the surrounding ovary tissue and immature oocytes as well as larger oocytes. This was required to get sufficient RNA yields, and is acceptable as the oocytes at this point do not produce their own RNA, and instead are fed RNA by surrounding ovary cells.
- Gene expression analysis shows *actb1* and *ef1a* as potential reference genes with some complications from uneven expression. Further testing will determine whether or not the variance is acceptable.
- Gene expression analysis for *ctnnb1* shows differential expression for both control and test groups, with a spike in expression at the four hour point. This raises the question of whether spontaneous maturation is occurring in the control group, or if there are other confounding factors.

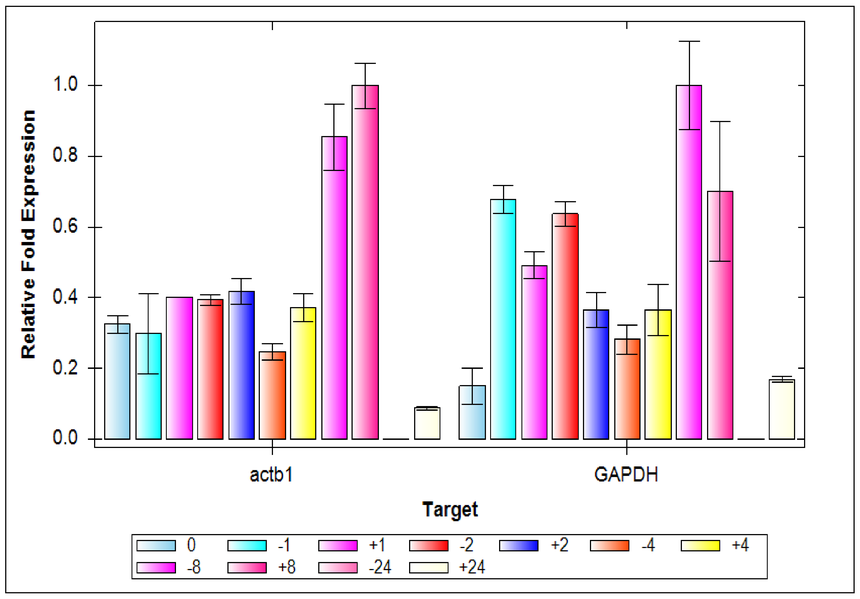

Figure 3. Gene expression for *actb1* and *gapdh* during maturation

Future Directions

- Continue work to define a reference gene for oocyte maturation, focusing on *actb1* and *ef1a*.
- Confirm if there are consistent and significant changes in *ctnnb1* expression during oocyte maturation.
- Examine changes in *dvl2* expression over time
- Examine the levels of Wnt signaling proteins during oocyte maturation, potentially through Western blotting.

Acknowledgements

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