

2010

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## Recommended Citation

Swinson, Julie P., "Time progression of Bub1 activation in Zebrafish (*Branchydanio rerio*) during egg maturation" (2010). *Summer Research*. Paper 12.  
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# Time progression of *Bub1* activation in Zebrafish (*Branchydanio rerio*) during egg maturation

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## Introduction

- The spindle assembly checkpoint, occurring before the second metaphase of meiosis, allows the cell undergoing cell division to check for DNA mutations and check cohesion between the kinetocores and meiotic spindle.<sup>1</sup>
- In Zebrafish egg maturation is triggered by the hormone progesterone, and several genes and proteins known as cytotstatic factors (CSF).<sup>1</sup>
- Resumption of meiosis is important for successful reduction of chromosomes prior to fertilization.
- CSFs are responsible for the inhibition of meiosis at the spindle assembly checkpoints; 6 genes are involved in the checkpoint; *mad1*, *mad2*, *mad3*, *bub1*, *bub3*, and *mps1*.<sup>2</sup>
- The CSF proteins are thought to be part of the MAP-K regulated pathway.<sup>3</sup>
- Bub1* is essential for activating the spindle assembly checkpoint to assure that the cohesion between kinetocores and the meiotic spindles is strong.<sup>4</sup>
- In studies where *bub1* has been inactivated the separation of chromosomes is a mess and shortly after the cell cycle arrests.<sup>5</sup>

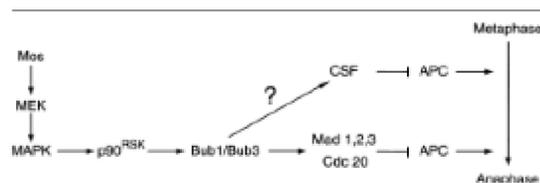


Figure 1. MAP-K regulated pathway of the spindle assembly checkpoint.

## Materials and Methods

- Follicle cell enclosed oocytes were collected from Zebrafish and treated with progesterone (P4) in ethanol or ethanol only (control)
- Oocytes were incubated at 26° C in 60% L-15 medium for various times from 0 hours to 12 hours
- Oocytes were removed from the culture medium and frozen. The sample was then lysed and processed through a series of centrifugations to collect the total RNA using a QIAGEN RNeasy mini kit
- Isolated RNA was reverse transcribed into the complementary DNA using the QIAGEN OneStep RT-PCR kit. This was done using random DNA primers in the reverse transcriptase buffer. The cDNA will be subjected to PCR using primers specific for the *bub1* and *EF1α* sequences
- The products of the RT-PCR reaction were run through a 1% agarose gel made with ethidium bromide for staining the DNA. The results were visualized under UV light
- Densities of the resulting bands were determined using the Image J software. Relative amounts of *bub1* transcript were then statistically analyzed to determine the amounts of *bub1* in response to progesterone during oocyte maturation
- All experiments were repeated in triplicate.

## Results

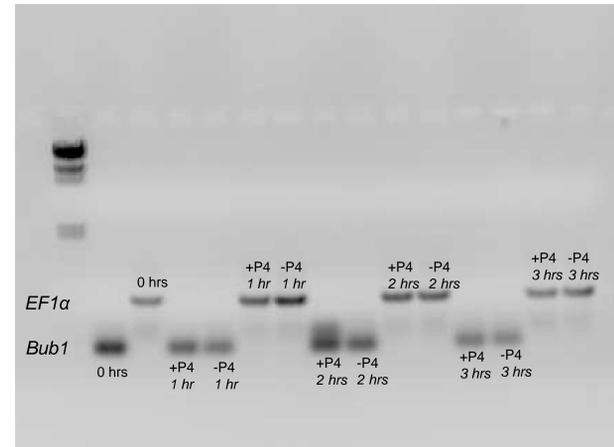


Figure 2. Expression of *bub1* in progesterone treated oocytes increases initially by hour 1, but decreases by hour 3. Expression of *EF1α* is constant between oocytes treated with progesterone and no hormone

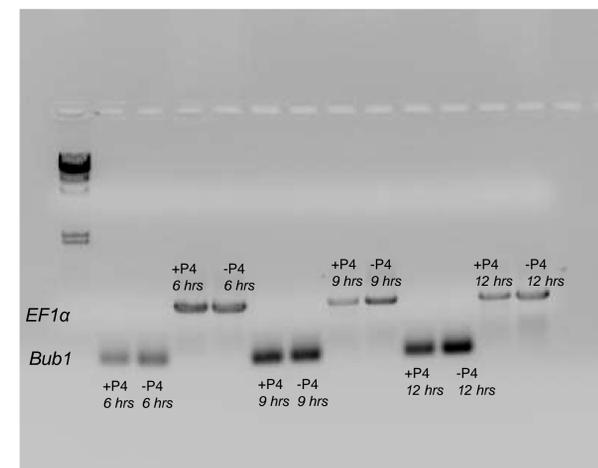


Figure 3. Expression of *bub1* increases from hours 6 to 12. Expression of *EF1α* is constant between oocytes treated with progesterone and no hormone

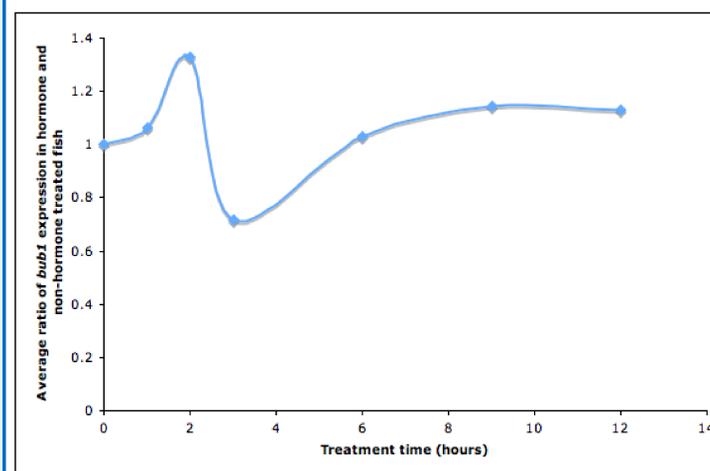


Figure 4. *Bub1* expression in hormone treated oocytes. *Bub1* expression initially increases, then decreases by hour 3, followed by an increase in expression over time after progesterone treatment.

## Conclusions

- Bub1* expression increases in Zebrafish follicle-cell enclosed oocytes shortly after progesterone stimulation, followed by a decrease in expression 3 hours after treatment.
- Bub1* expression increases constantly after three hours of treatment with progesterone.
- Bub1* plays an important role in oocyte maturation in Zebrafish during the resumption of meiosis

## Future Research

- Explore possible explanations for decrease in *bub1* RNA expression 3 hours after treatment with progesterone
- Explore possible differences in *bub1* activation between live-bearing fish and egg-bearing fish
- Examine gene activation of other cytotstatic factors, such as *Sgo1*, which is closely associated with *bub1* and the cohesion between the kinetochores and spindle
- Identify if *bub1* is activated in the egg and/or the follicle cells of Zebrafish oocytes

## Acknowledgments

University of Puget Sound Biology Department  
 Dr. Alyce DeMarais  
 Dr. Mark Martin  
 Michal Morrison-Kerr  
 Mandi Wood and Min Lee

## References

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