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The Time Progression of Bub1 and Cdc2 activation in zebrafish (Danio rerio) during egg maturation

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The time progression of *Bub1* and *Cdc20* activation in zebrafish (*Danio 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.[1]

•In Zebrafish oocytes maturation is triggered by the hormone progesterone, and several genes and proteins known as cytostatic factors (CSF).[1]

•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; some of the many genes involved in this checkpoint are bub1, mad1 and mps1, a signal transducer known as the MCC (mitotic checkpoint complex), which is made up of bubR1, bub3, mad2 and cdc20.[2]

•*Bub1* is essential for activating the spindle assembly checkpoint to assure that the cohesion between kinetocores and the meiotic spindles is strong.[2]

•*Bub1* was essential in the proliferation of cells and that shortly after *bub1* inactivation in embryos their development was arrested.[2]

•*Cdc20* is essential for the activation of the APC (ananphase promoting complex) and the resumption of meiosis.[3]

•*Cdc20* depletion causes an increase in aneuploid cells, indicating that the presence of *cdc20* is somehow connected to chromosome separation and segregation.[4]

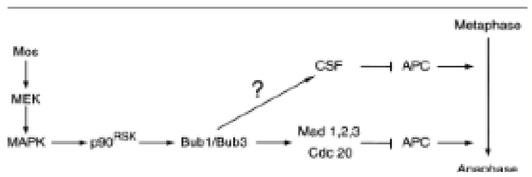

Figure 1. MAP-K regulated pathway of the spindle assembly checkpoint.[5]

References

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Results

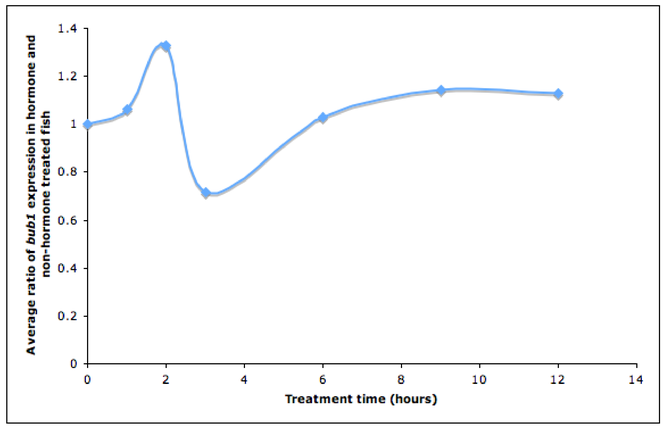

Figure 2. *Bub1* expression in hormone treated oocytes. Initial analysis of *Bub1* expression showed an initial increase, then decrease by hour 3, followed by an increase in expression over time after progesterone treatment.

- Initial analysis demonstrated an inverse relationship between *bub1* expression and *cdc20* expression in Zebrafish oocytes
- RT-PCR preformed on oocytes treated with nocodazole were inconsistent and inconclusive
- RT-PCR trials run towards the end of the summer indicated amplification of *bub1* and *cdc20* genes was not successful in experimental trials
- Implications of this include incorrect data analysis and misunderstood results
- Possible causes of unsuccessful amplification include incorrect measurement of RNA concentration in samples, and lack of primer recognition.

Conclusions

•*Bub1* expression increases and decreases in Zebrafish follicle-cell enclosed oocytes durring the 12 hours folowing DHP treatment.
•Expression of *Bub1* and *cdc20* genes following treatment with DHP have an inverse relationship.
•Following treatment with nocodazole, expression of *bub1*, *cdc20* and *ef1 alpha* were all affected and demonstrated the inverse relationship between *bub1* and *cdc20.*
•*Bub1* and *cdc20* play an important role in oocyte maturation in Zebrafish during the resumption of meiosis following the spindle assembly checkpoint

Materials and Methods

•Follicle cell enclosed oocytes were collected from Zebrafish and treated with DHP (hormone) in ethanol or ethanol only (control)
•Some oocytes were subjected to nocodazole treatment in order to understand the effect of nocodazole on *bub1*, *cdc20,* and *EF1 alpha* expression
•Oocytes were incubated at 26˚ C in 60% L-15 medium for various times from 0 hours to 24 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
•RNA concentration was measured using the Quibit flourimeter in order to determine the amount of RNA needed for the RT-PCR reaction
•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, cdc20* 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* and *cdc20* transcript were then statistically analyzed to determine the amounts of *bub1* and *cdc20* in response to DHP during oocyte maturation
•All experiments were repeated in triplicate.

Future Research

•Design primers that recognize *bub1* and *cdc20* genes and increase amplification
•Explore possible explanations for increase and decrease in *bub1* and *cdc20* RNA expression following treatment with DHP
•Explore possible differences in *bub1* activation between live-bearing fish and egg-bearing fish
•Examine gene activation of other cytostatic factors
•Identify if *bub1* and *cdc20* is activated in the egg and/or the follicle cells of Zebrafish oocytes
•Determine the effect of nocodazole treatment on RNA expression of *bub1, cdc20* and *ed1 alpha* in oocytes

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