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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 kinetochores 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 kinetochores 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 (anaphase 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

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.5

Initial analysis demonstrated an inverse relationship between bub1 expression and cdc20 expression in Zebrafish oocytes.

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 fluorimeter 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.

References


Results

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

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.

Conclusions

Bub1 expression increases and decreases in Zebrafish follicle-cell enclosed oocytes during the 12 hours following 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.

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 ef1 alpha in oocytes

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