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Zebrafish (Danio rerio) oocyte maturation and development

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Background

•During development, the oocyte has signalling mechanisms that help promote the fidelity of chromosome segregation during meiosis.
•The spindle assembly checkpoint will halt the cell cycle if chromosomal abnormalities are present, in order to promote repair mechanisms (Tunquist and Maller, 2003)
•The signalling cascade is thought to be triggered by abnormalities in the spindle apparatus

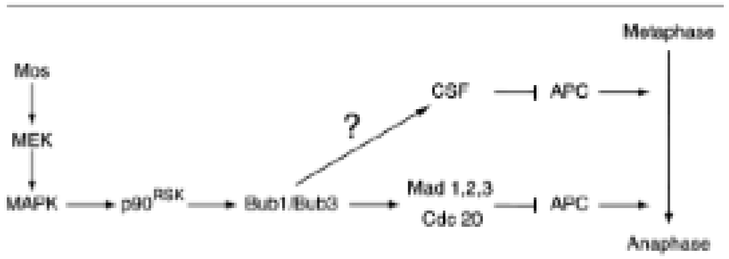

Figure 1. The current molecular model for mitotic checkpoint activation.(Tunquist and Maller, 2003)

•Follicle cells provide nutrients and transmit signals to the developing ovum for growth and maturation
•Studies of checkpoint proteins in oocytes should be corresponded with studies of follicle cells

Present Study

•We have asked whether prematurational oocytes respond to a signaling cascade destabilizing treatment by upregulating the expression of genes found in the spindle assembly checkpoint (specifically, *Bub-1*)
•We have also asked whether it be possible to detect chromosomal or nuclear morphological data from the follicular cells themselves.

Materials and Methods

•PART I: expression of *Bub-1* under altered oocyte conditions
•Oocytes were treated with microtubule destabilizing drug, Nocadozole (Ikegami et al., 1997).
•Total RNA was extracted from treated oocytes
•*Bub-1* gene transcripts were amplified by RT-PCR, with EF1-α sequences also amplified as a loading control.
•Densitometry analysis was performed on the resulting PCR products using ImageJ to determine statistical power

PART II: developing a protocol for visualization of follicular nuclei
•Oocytes were fixed for 2 hours using a new "fixing solution" (100mM HEPES, 50mM EGTA, 5mM MgSO4, 0.4 M Dextrose, 0.2% Triton X-100, 32% Formaldehyde, and 1x PBS in water) and washed in PBTriton 0.1%
•Nuclear material was stained with Hoechst 33342
•Follicle cell nuclei were visualized on a fluorescent microscope at 20x magnification.

Results

•The Bub-1 sequence is up-regulated in oocytes treated with Nocadozole.

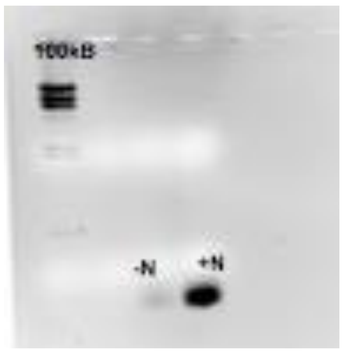

Figure 2. Agarose gel of *Bub-1* RT-PCR products. Densitometry reveals that cells treated with Nocadozole expressed *Bub-1* significantly more than the control. (p=0.0295, 95% confidence interval 166.26, 1192.06, n=6)

•Follicle cell nuclei are visible under fluorescent microscope

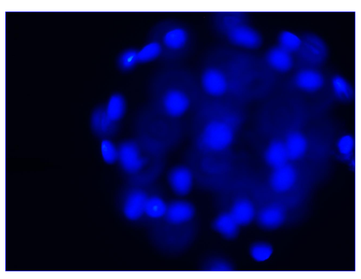

Figure 3. Fluorescent microscope image (20x) of follicle cell and oocyte in egg culture stained with Hoescht 33342.

Conclusions

•The oocyte mitotic process is sensitive to cellular condition and treatments
•The oocyte responds to microtubule destabilization by up-regulating checkpoint protein *Bub-1*

•Follicular nuclei can be successfully visualized from oocyte culture using the newly-developed protocol

Future Research

•Connect the variation in oocyte condition with that of the follicle cells.
•Immunofluorescent studies of follicle cells in conjunction with the checkpoint proteins
•More gene expression assays should be performed with other genes in the pathway

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References

1. Tunquist, B.J. and Maller, J.L. 2003. Under arrest: Cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.* 17: 683-710.

2. Ikegami, Richard; Zhang, Jianshe; Rivera-Bennetts, Alma K.; Yager, Thomas D.; 1997. Activation of the metaphase checkpoint. *Zygote*. 5: (November) 329-350.