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Analysis of Mos-MEK-MAPK Pathway during Egg Maturation in *Athalia rosae ruficornis* (Hymenoptera)*

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Egg meiosis arrests before fertilization, and it resumes upon fertilization (or egg activation). Arrest occurs at the metaphase of meiosis II (MII) in vertebrate eggs, while it occurs at the metaphase of meiosis I (MI) in insect eggs. It has been demonstrated that the MII arrest of vertebrate eggs is regulated by the Mos-MEK-MAPK pathway (Tunquist and Maller, 2003). However, the mechanisms of MI arrest remain unclear for the most part including the participation of the Mos-MEK-MAPK pathway. In the sawfly, *Athalia rosae ruficornis* (Hymenoptera), unfertilized eggs can easily be activated *in vitro*, and thus egg meiosis can be induced to resume artificially. We have been analyzing the mechanisms of MI arrest using *A. rosae* as a model organism (Yamamoto *et al.*, 2004, 2005).

We have cloned the *c-mos* gene orthologue of *A. rosae* (*Armos*) encoding Mos protein and shown the gene is expressed in ovaries by RT-PCR (Yamamoto *et al.*, 2005). Detailed expression analysis was performed with *in situ* hybridization using RNA probes. *Armos* mRNA was detected in nurse cells and eggs. The results indicate that *Armos* is transcribed in nurse cells, and the transcripts are accumulated in maturing eggs as a maternal gene product. Mos protein was present in mature eggs in which meiosis arrested at MI, and it disappeared when meiosis resumed.

Biochemical examination revealed that MEK and MAPK were activated through phosphorylation in mature, MI-arrested eggs in the presence of Mos. Injection of the GST-fusion *A. rosae* Mos protein into embryos, in which meiosis had resumed and MEK and MAPK were inactive, activated MEK and MAPK again. The results show unequivocally that Mos is the upstream regulator of MEK and MAPK. To examine whether MEK regulates MAPK activity, mature eggs were treated with the MEK inhibitor, U0126. MAPK began dephosphorylating within 2 h of U0126 treatment and lost its activity. Combining the present results together, the Mos-MEK-MAPK pathway participates in and plays a central role in MI arrest in *A. rosae*.

We confirmed the cytostatic activity of *A. rosae* Mos by biological examinations. Injection of the GST-fusion *A. rosae* Mos protein into *A. rosae* embryos inhibited further embryonic development. When the GST-fusion *A. rosae* Mos protein was injected into one blastomere of the two-cell-stage embryo of *Xenopus laevis*, the cell cycles were affected and the cleavage divisions ceased but only in the injected blastomere.

References

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