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Cytological Analysis of the CSF Function in *Athalia rosae ruficornis* (Hymenoptera)*

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In animal eggs, the meiotic cell cycle is normally arrested before fertilization and resumes upon egg activation, usually fertilization. In vertebrate eggs, the meiotic cell cycle arrest occurs at metaphase of meiosis II (MII). It has been clearly demonstrated that the Mos-MEK-MAPK pathway plays an essential role as a cytostatic factor (CSF) in MII arrest (Tunquist and Maller, 2003; Liu *et al*., 2007). In eggs of many invertebrates including insects, however, the meiotic cell cycle is arrested at metaphase of meiosis I (MI). The molecular mechanisms of MI arrest remain unclear for the most part, because it is technically difficult in these organisms except for some marine invertebrates to artificially induce egg activation (or fertilization) *in vitro*.

We have been analyzing the mechanisms of MI arrest using the hymenopteran insect *Athalia rosae ruficornis*, in which egg activation can be induced artificially. We have shown through biochemical analyses that the Mos-MEK-MAPK pathway participates in MI arrest (Yamamoto *et al*., 2005, 2006). MEK and MAPK are inactive in immature oocytes, while they become active in mature, MI-arrested eggs. The activities of MEK and MAPK decrease upon egg activation, and are completely lost within 60 minutes. When the GST-fusion *A. rosae ruficornis* Mos protein (GST-ArMos) is injected into these eggs, MEK and MAPK are re-activated. Treatment of MI-arrested eggs with the MEK inhibitor, U0126 results in inactivation of MAPK.

Here, we investigated the relationship between activities of MEK and MAPK and cell cycle progression. Progression of meiotic cell cycles after egg activation, and the status of nuclear (syncytial) divisions in eggs that receive GST-ArMos injection or treatment of the MEK inhibitor were examined cytologically by staining chromosomes with DAPI (4',6-Diamidino-2-phenylindole). MI arrest was released upon egg activation and meiosis proceeded to anaphase I after 20-minute egg activation, progressing to metaphase II and anaphase II after 40 and 60 minutes, respectively. Meiosis completed after 90 minutes. Female pronucleus was formed after 120 minutes. In eggs injected with GST-ArMos when MEK and MAPK activities were completely lost (50 minutes after egg activation), syncytial divisions ceased after a certain number. MI arrest was released and meiosis completed in eggs that received treatment of the MEK inhibitor. These observations well agreed with the status of MEK and MAPK activities, and further support our earlier notion that the Mos-MEK-MAPK pathway participates in MI arrest of *A. rosae ruficornis* eggs.

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