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Article / Book Information

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論 文 要 旨

THESIS SUMMARY

専攻： Department of	Biological Information 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 (Science) Doctor of
学籍番号： Student ID Number		指導教員（主）： Academic Advisor(main)	Tachibana Kazunori
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In most animals, fully grown oocytes are arrested at G2 phase of meiosis I (immature oocyte). In these immature oocytes the germinal vesicle (GV) is positioned at the center (mammals) or close to the animal pole (echinoderms, amphibians and fish). Upon hormonal stimulation, oocyte maturation begins, germinal vesicle breakdown (GVBD) occurs, and small polar bodies are extruded from large oocytes by asymmetric spindle localization [polar body extrusion (PBE)]. After GVBD, the spindle forms, moves and attaches to the cortex, and the cleavage furrow forms around the spindle, resulting in PBE. Movement of the spindle to the cortex has been well studied in mouse oocytes. However, the maintenance of spindle attachment to the cortex has not been studied and cleavage furrow formation during PBE needs further clarification.

The Mos-MAPK-p90Rsk pathway is a meiosis specific cell cycle regulatory pathway. It is required for transition from meiosis-I to meiosis-II, and for the secondary meiotic arrest (G1 arrest in starfish oocytes). Several studies suggest that the Mos-MAPK pathway is required for PBE; however its role during PBE remains unclear.

Diaphanous-related formins (Dia) are a subgroup of formins which are basically responsible for straight F-actin formation, and there are three Dia proteins in mammals: Dia1, Dia2 and Dia3. Dia proteins are required for proper spindle formation, actin tubulin organization, cytokinesis and microtubule–kinetochore attachment. The budding yeast Diaphanous-related formins, Bni1 and Bnr1, are important for unequal cell division; however, roles of Dia proteins in PBE have not been studied. Dia proteins have a

Dia autoregulatory domain (DAD) at the C-terminus and a DAD interacting domain (DID) at the N-terminus, and DID overlaps with the GTPase-binding domain (GBD). DAD and DID interact with each other and thereby keep Dia inactive. Small-GTPase binding to GBD enhances Dia activation but is not sufficient to fully activate Dia in physiological conditions.

In this study, first of all, I have concentrated on whether the Mos-MAPK pathway is required for PBE in starfish oocytes or not. For this purpose, immature oocytes were injected with antisense oligonucleotide against Mos (MosAS) to prevent Mos synthesis or treated with a MEK-specific inhibitor, U0126, to prevent MAPK activity. To visualize spindle and chromosome dynamics in these oocytes, immature oocytes were injected with fluorescent labeled histone and tubulin proteins, and then the maturing oocytes were observed with live-cell imaging. In contrary to control oocytes, MosAS injected oocytes or MAPK-inhibited oocytes failed in PBE; in these oocytes the spindles were not kept tightly attached to the cortex, chromosomes were not segregated properly, and finally, although a membrane protrusion for a polar body was formed, the cleavage furrow failed to close. Next, immature oocytes were injected with a constitutively active mutant of p90Rsk (CA-p90Rsk) or a kinase dead mutant (KD-p90Rsk), and treated with U0126. CA-p90Rsk was able to recover all the defects caused by U0126-treatment while KD-p90Rsk was not. This latter observation, suggests that p90Rsk might be performing the functions of MAPK during PBE. Thus, I have shown that the Mos-MAPK-p90Rsk pathway is required for PBE in starfish oocytes.

Since there was a defect in cleavage furrow closure, I have concentrated on the regulation of the F-actin mechanisms to find a downstream target of the Mos-MAPK-p90Rsk pathway. Diaphanous-related formins (Dia) are actin nucleating factors that are known to be required for contractile ring formation at the cleavage furrow during mitotic division and during budding of the yeast. Treatment of oocytes with a formin specific inhibitor, SMIFH2, prevented cleavage furrow closure during PBE.

Based on this, *Asterina pectinifera* Dia (ApDia) was cloned, and then the oocytes expressing GFP2-ApDia was monitored during PBE; GFP2-ApDia had a ring like localization at the neck of the protrusion, and its localization was altered in MAPK-inactivated oocytes. To analyze whether activity of ApDia was also under the control of MAPK, in the absence of MAPK activity, endogenous ApDia was activated by injecting the immature oocytes with GST-DAD (GST-Dia autoregulatory domain) or GST-GBD/DID (GST-GTPase binding domain/DAD interacting domain) peptide fragments, and this activation recovered all defects caused by U0126-treatment. Finally, expressing a constitutively active form of ApDia (ApDia-fsDAD) rescued PBE in the absence of MAPK-activity whereas an actin binding mutant (ApDia-fsDAD-I699A) did not.

To sum up, I have shown in this study that, the Mos-MAPK-p90Rsk pathway is required for PBE by maintaining tight spindle attachment, proper chromosome separation and cleavage furrow closure. Additionally, I have shown that localization of ApDia to the cleavage furrow and its activation is being regulated by the activity of the Mos-MAPK pathway and that in MEK/MAPK-inhibited oocytes, activation of ApDia by the relief of its intramolecular inhibition restores PBE. In summary, this study elucidates a link between the Mos-MAPK pathway and diaphanous-related formins, that is responsible for maintaining tight spindle attachment to the cortex and cleavage furrow closure during PBE.