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THE CONTENT OF GERMINAL VESICLE IN FROG OOCYTES INHIBITS AN ACTIVATION OF MPF.

Sadakane, Y. and Iwao, Y.
Biol. Inst., Fac. Sci., Yamaguchi Univ., Yamaguchi, Japan

Amphibian oocytes have been arrested at prophase I during oogenesis. To understand the mechanism of the arrest at the specific stage of cell cycle, we have investigated whether the factor that inhibits progress of the cell cycle exists in the frog, Rana rugosa, oocytes, especially germinal vesicles (GVs). Injection of GV-content into fertilized Cynops eggs prevented them from cleaving up to 12hr after fertilization when uninjected eggs reached at 16-cell stage. Nuclear division in zygote nuclei was stopped at prophase (G2-like stage), and rising of MPF activity was not detected. Furthermore, re-injection of MPF-fraction into the arrested eggs was able to resume the cleavage. The factor was heat-stable ($50^{\circ}C$, 10 min) and was not sensitive to Ca^{2+} and Mg^{2+}. The ultrafiltration indicates that MW of the factor is more than 10^6. When the GV-content had been injected into the full-grown Bufo oocytes, GV break down was not induced by progesterone, but that was induced by MPF-injection. These results suggest that the GV has the factor that inhibits an activation of MPF, which might ensure the prophase-arrest in growing frog oocytes.

THE ROLE OF EGG-NUCLEUS ON CELL CYCLE AND BEHAVIOR OF SPERM NUCLEI IN THE PHYSIOLOGICALLY POLYSPERMIC NEWT EGGS.

Yasuhiro Iwao and Yuki Myôtoishi
Inst. Biol., Fac. Sci., Yamaguchi Univ., Yamaguchi

It has been demonstrated that the removal of egg-nucleus in the fertilized newt egg induces rescue of accessory sperm nuclei. To determine whether maturation promoting factor (MPF) involves in this process, we investigated that the cycle of MPF activity as well as the behavior of sperm nuclei in the eggs whose egg-nuclei at MII had been removed with a micropipet or UV-irradiation at MII just after fertilization. The MPF activity had been maintained for longer period than that in normal eggs, so that the onset of multipolar cleavage by the undegenerating accessory sperm nuclei was delayed for 2 hr. Most of the sperm nuclei in the animal hemisphere entered M-phase completely, but the rest in the vegetal one degenerated. These results support our hypothesis that the high activity of MPF around the zygote nucleus ensure to complete the mitosis, and the accessory sperm exposed by lower MPF activity can not complete it. The delay of cell cycle in the UV-irradiated eggs indicated that the egg nucleus has predominant activity for both entering and completing M-phase which might be controlled by molecules concerning $S\text{-}G_2(M)$ transition, such as CDC 25 or RAD1.

CHROMOSOME CONDENSATION WITHOUT PROTEIN SYNTHESIS IN UNFERTILIZED SEA URCHIN EGGS INDUCED BY CALYCULIN A.

H. Tosuji[1], I. Mabuchi[2] and T. Nakazawa[3].
[1]Dept. of Biol., Fac. of Sci., Kagoshima Univ., Kagoshima, [2]Dept. of Biol., Coll. of Arts & Sci., Univ. of Tokyo, Tokyo and [3]Dept. of Biol., Fac. of Sci., Toho Univ., Funabashi.

It has been considered that two proteins, cdc2 kinase and cyclin B, form a complex and regulate the cell cycle in eukaryotic cells as the M-phase promoting factor (MPF). While the former protein exists throughout the cell cycle, cyclin B accumulates during the interphase up to metaphase and then disappears abruptly at the end of metaphase in each cell cycle. Calyculin A is known to inhibit activities of protein phosphatase type-1 and -2A. Condensation of chromosomes was observed in unfertilized sea urchin eggs when they were incubated with calyculin A. An increase in histone H_1 kinase activity was observed in extracts from these eggs. No protein synthesis was detected in these eggs during the calyculin A treatment. Moreover, these phenomena were observed in the presence of emetine, an inhibitor for protein synthesis. These results suggest that MPF is activated by the calyculin A treatment without protein synthesis. However, it has been reported that the unfertilized sea urchin eggs have no pool of cyclin B. Therefore, the calyculin A activation of cdc2 kinase could have occurred independently of cyclin B.

INDUCTION OF NUCLEAR DECONDENSATION OF AND PROTAMINE REMOVAL FROM HUMAN SPERM BY NUCLEOPLASMIN.

T. Itoh[1], K. Ohsumi[2], and Ch. Katagiri[1].
[1]Zool. Inst., Fac. Sci., Hokkaido Univ., Sapporo, [2]Lab. Cell and Dev. Biol., Tokyo Inst. Technol., Tokyo.

Previous studies showed that the lysolecithin (LC)-dithiothreitol (DTT)-treated human sperm nuclei undergo decondensation and develop into pronuclei when incubated in the egg extracts from *Xenopus laevis* or *Bufo japonicus*. Starting with 150kxg supernatants from *Bufo* egg extracts, the activity inducing decondensation of LC-DTT-treated human sperm was fractionated as 0.4-0.5 M KCl eluants by anion-exchange chromatography on Q-Sepharose, followed by gel-filtration on Superose 12 as a broad peak at 200-400 kDa. When LC-DTT-treated sperm were incubated in this 200-400 kDa fraction, the resulting supernatant was found to contain protamines on acetic acid/urea/Triton X-100 (AUT)-PAGE. Because of the similarity in properties of this fraction with those of nucleoplasmin, we incubated LC-DTT-treated human sperm nuclei with nucleoplasmin that was prepared according to the protocol by Sealy *et al.* (1986) for *Xenopus*. It resulted that both decondensation of and protamine removal from human sperm nuclei were induced by nucleoplasmin from *Bufo* eggs.

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