SELECTIVE DESTRUCTION OF ACTIN-BASED CYTOSKELETON NETWORKS IN THE IMMATURE STARFISH OOCYTE BY THEONELLAPEPTOLIDE IE, A PEPTIDE FROM A MARINE SPONGE

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Theonellapeptolide Ie (Tp), an oligopeptide lactone isolated from a marine sponge, *Petrosia* sp., was shown to induce an unprecedent morphological change in the immature oocyte of the starfish, *Asterina pectinifera*. The cortical F-actin was disturbed and assembled to form dots and rings, as evidenced by staining with rhodamine-conjugated phalloidin. The oocyte eventually became malformed. When Tp was added to an immature oocyte which had been pretreated with cytochalasin B or D, inhibitors of actin polymerization, no malformation was observed. When Tp was added to an oocyte which had been induced to mature by 1-methyladenine (1-MeAde), a maturation-inducing substance in starfishes, the percentage of malformed oocytes gradually decreased corresponding to the period of 1-MeAde-pretreatment, and no malformation was observed in the mature eggs. These results suggest that Tp-sensitive factors closely relate to the stabilizing component of F-actin-based cytoskeleton in an immature oocyte but disappear during maturation.

MATERAL mRNAs THAT ARE LOCALIZED TO MYOPLASM IN ASCIDIAN EGG ASSOCIATE WITH CORTIAL ENDOPLASMIC RETICULUM

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In ascidians, maternal mRNAs such as macho-1, a determinant of muscle cell fate, belong to Type I postplasmic RNAs located in myoplasm of eggs. Prior to the first cleavage these postplasmic RNAs relocate in 2 main phases. They further concentrate into a cortical structure, the centrosome-attracting body (CAB), during early cleavages. By using high-resolution fluorescent *in situ* hybridization in Halocynthia roretzi, we showed that macho-1 and HrPEM are localized on a reticulated structure situated within 2 μm of the unfertilized egg cortex, and within 8 μm of the cortex of the vegetal region and then posterior region during ooplasmic segregation. By isolating cortices from eggs and zygotes we demonstrated that this reticulated structure is a network of cortical rough endoplasmic reticulum (cER) tethered to the plasma membrane. We also showed that macho-1 and HrPEM accumulate in the CAB together with the cER network. We propose that these postplasmic RNAs relocalize after fertilization by following the microfilament- and microtubule-driven translocations of the cER network. We also suggest that the RNAs concentrate in the CAB through compaction of the cER to form the CAB.

INVOLVEMENT OF cAMP/PKA PATHWAY DURING INITIATION OF OOCYTE MATURATION IN THE OVARY OF HYDROZOAN *CYTAEIS UCHIDAE*

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In medusae of hydrozoan *Cytaeis uchidae*, the trigger for oocyte maturation is the light cue after the dark period. The oocyte maturation proceeded within the ovaries, and the matured oocytes are released from the ovaries. On the other hand, the continuous increase in intracellular cAMP induced oocyte maturation in isolated oocytes from the ovary without light stimulation. This process was inhibited by the specific blockers of protein kinase A (PKA). In this study, we examined whether the cAMP/PKA pathway is actually involved in light-triggered physiological meiosis reinitiation, which progresses inside the ovaries. When the ovarian oocytes just beneath the epithelium were injected with the PKA inhibitor (H-89 or Rp-cAMPS) within 3 minutes after dark-light transition, the oocyte maturation was inhibited or delayed, while oocytes injected about 5 minutes after dark-light transition and non-injected oocytes in the same medusae underwent germinal vesicle breakdown on schedule. These results suggest that cAMP/PKA pathway has an important role for initiation of oocyte maturation of *C. uchidae in vivo*.

KARYOPLASM DIFFUSION DURING GERMINAL VESICLE BREAKDOWN IN STARFISH OOCYTES PROCEEDS IN TWO PHASES

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The process of germinal vesicle breakdown (GVBD) in starfish oocytes was analyzed in detail using time-lapse video microscopy. In *Astropecten scoparius* oocytes, diffusion of karyoplasm was found to slow down tentatively during 4 - 7 min after the onset of GVBD. Cytochalasin B (CB) treatment delayed the diffusion only after that slowdown period. Although the slowdown period was not detected in *Asterina pectinifera* oocytes, CB treatment particularly delayed later process of GVBD as in the case of *Asteropecten* oocytes. These results suggest that GVBD process in starfish oocytes consists of two phases, i.e., the first CB-insensitive and the second CB-sensitive phase. Later CB-sensitive phase may represent actin-enhanced breakdown of the nuclear lamina, which is known to remain until later process of GVBD. Colchicine, or nocodazole had little effect on GVBD, while actin-stablizing agent, jasplakinolide, delayed overall process of karyoplasm diffusion in *Asterina* oocytes. These results indicate that actin cytoskeleton is involved in GVBD process in starfish oocytes.

THE NON-UNIFORM DISTRIBUTION OF NOVEL DNA AMONG THE CENTRIOLES OF THE MEIOTIC SPINDLES IN OOCYTES OF *ASTERINA PECTINIFERA*

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We discovered novel DNA in the sperm centrosome of the starfish *Asterina pectinifera*. The nucleotide sequence of the DNA is different from that of the mitochondrial DNA of this species (2002, Kawai). In starfish oocytes, maternal centrosomes are not equivalent in reproductive capacity. Only one of the two centrosomes of a meiosis II spindle, which is destined to be cast off into the second polar body, retains reproductive capacity, while both of the centrosomes forming a meiosis I spindle are reproductive. Hence, the first and the second polar body(PB1, PB2) have a reproductive centrosome, and the mature egg inherits a nonreproductive one. To detect the novel DNA in those centrosomes by whole mount *in situ* PCR, we used a specific primer (CS primer) prepared for the sperm centrosomal DNA. The DNA was detected in each of the two centrosomes forming the meiosis I spindle as well as in mitotic spindles. In contrast, a positive signal was detected in only one pole of the meiosis II spindle. Both PB1 and PB2 showed the evidential signal for the novel DNA. Our results clearly show that the novel DNA is localized only in the centrosomes with reproductive capacity.

P90 RSK IS REQUIRED FOR G1-ARREST IN UNFERTILIZED STARFISH EGGS

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Mos, a main component of CSF (cytostatic factor), causes Meta-2 -arrest in frog eggs and G1-arrest in starfish eggs. Although p90 Rsk mediates the Mos-MAPK function as CSF in frog, the downstream mediator of Mos-MAPK in starfish is unknown. To investigate whether p90 Rsk is involved in G1-arrest in starfish, we have cloned starfish homolog of p90 Rsk and raised its antibody. p90 Rsk was activated after GVBD (germinal vesicle breakdown) and inactivated after fertilization. In immature oocytes, p90 Rsk was activated by microinjection of GST-Mos. In Meta-1 oocytes, p90 Rsk was inactivated by MEK inhibitor U0126. When immature oocytes received microinjection of the neutralizing antibody against p90 Rsk and then underwent meiotic maturation, DNA replication was observed in egg pronuclei in the absence of fertilization. These results show that p90 Rsk is a downstream mediator of the Mos-MAPK pathway and required for G1-arrest in unfertilized starfish eggs.

EFFECTS OF POLYSPERMY ON POLAR BODY FORMATION

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Fully grown starfish oocytes are arrested at prophase of the first meiosis. The hormonal stimulation of 1-methyladenine (1-MA) induces meiosis reinitiation, and germinal vesicle breakdown (GVBD) occurs. Optimal development occurs when maturing oocytes are fertilized between GVBD and the first polar body emission. The first polar body is released at 80-90 min after 1-MA treatment. When immature oocytes are inseminated, polyspermy occurs readily. Subsequent treatment of inseminated oocytes with 1-MA induces GVBD and a high incidence of abnormal embryonic development.

In this study, we found that the oocytes with numerous incorporated sperm released 2-3 polar body-like cleaved fragments (PBCF) 80-90 min after 1-MA treatment. When the polymerization of cortical actin was suppressed by cytochalasin B, PBCF did not form. We did not find chromosomes and asters in PBCF. Thus, extrusion of PBCF occurs without MTOC.