THE DEPENDENCE OF MEIOSIS RESUMPTION UPON INTRACELLULAR PH AND CALCIUM IN THE OOCYTES OF THE MARINE BIVALVE LIMARIA HAKODATENSIS.

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The oocytes of Limaria hakodatensis (Mollusca, Pelecypoda) in the ovary remain arrested at prophase-I (PI), whereas the spawned oocytes are arrested at metaphase-I until fertilization. When the PI-arrested oocytes, injected with pH-dependent fluorescent probe HPTS (8-hydroxy-1,3,6-pyrenetrisulfonate), were exposed to NH_4Cl-seawater, intracellular pH (pH_i) rose rapidly and germinal vesicle breakdown (GVBD) occurred. In order to induce GVBD, pH_i above the threshold had to be maintained for some minutes. In the MI-arrested oocytes injected with fluorescent Ca-indicator fura-2, intracellular free Ca^{2+} (Ca^{2+}_i) increased transiently after fertilization. It was followed by repetitive Ca^{2+}_i spike oscillation. Ionomycin, which also activates MI-arrested oocytes, caused not Ca^{2+}_i oscillation but constant Ca^{2+}_i increase. A Ca chelator BAPTA-AM inhibited both sperm- and ionomycin-induced activations. These data indicate that pH_i rise is necessary for the release from PI, whereas Ca^{2+}_i increase is responsible for the release from MI. Furthermore, The repetitive Ca^{2+}_i spike oscillation after fertilization was also seen in the oocytes of Mytilus edulis and Crassostrea gigas, so this phenomenon seems to be universal in bivalves.

SOLUBILIZATION OF 17α,20β-DIHYDROXY-4-PREGNEN-3-ONE-BINDING ACTIVITY FROM PLASMA MEMBRANE FRACTION OF TROUT OOCYTES.

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We reported that plasma membrane fractions prepared from oocytes of rainbow trout specifically bound [^{3}H]17α,20β-DIHYDROXY-4-PREGNEN-3-ONE ([^{3}H]17α,20β-DP). This activity has been successfully solubilized from the membrane preparations with anionic detergents, sucrose monolaurate and *n*-heptyl-β-D-thioglucoside. Hydroxyapatite was used to separate bound from free ligand. In agreement with the previous experiment with membrane preparations, binding of [^{3}H]17α,20β-DP to solubilized samples was saturated within one hour, and competitively inhibited with an excess amount of non-radioactive 17α,20β-DP. Unexpectedly, estradiol-17β also inhibited the binding.

We also identified and purified a specific binding protein for 17α,20β-DP (DBP) in trout blood plasma. Purified DBP showed a strong affinity (Kd=8nM) to 17α,20β-DP with an apparent molecular size of 110 kDa on native-PAGE and 50 and 55 KDa on SDS-PAGE. Preincubation of oocyte plasma membrane preparations with flesh DBP enhanced 17α,20β-DP binding to the membrane preparations. After DBP was stored for 3 days at 4°C, its enhancing activity was lost without losing its 17α,20β-DP binding activity. EDTA inhibited the enhancing activity of DBP. DBP may play a role in enhancing 17α,20β-DP binding to oocyte plasma membranes.

IDENTIFICATION OF SEROTONIN AS A LOW MOLECULAR-WEIGHT SERUM FACTOR THAT INDUCES IN VITRO MATURATION OF THE MEDAKA OOCYTE.

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A low molecular-weight factor that induces in vitro maturation of intrafollicular oocytes of Oryzias latipes was isolated and purified from chicken blood by means of dialysis, and HP-20 and reversed phase HPLC. The serum factor was purified by repeated fractionations on HPLC using a solvent with a linear gradient trifluoroacetate, with detection at UV-215nm. The HPLC-purified substance which induced in vitro oocyte maturation was identified as serotonin (5-HT) by three different criteria: (1)Characteristic HPLC retention time, (2)Comparison of UV-spectra and (3)NMR analysis. Serotonin induced in vitro maturation of intrafollicular oocytes at concentrations >45nM but exerted no effect on defolliculated oocytes. Additionally, serotonin stimulated the in vitro production of steroids in the medaka follicular cells. The data suggest that serotonin as a low molecular-weight serum factor exerted its action on in vitro maturation of fish oocytes via steroids produced by follicular cells.

RELOCATION OF MPF:CDC2/CYCLIN B DURING MEIOSIS REINITIATION IN STARFISH OOCYTES.

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Using indirect immunofluorescence staining with anti-starfish cyclin B antibody, we found that inactive MPF is exclusively present throughout the cytoplasm but not in the germinal vesicle of immature starfish oocytes. After its activation, a part of MPF moved into the germinal vesicle just before the onset of its breakdown and accumulated into nucleolus and condensing chromosomes. Other part of MPF accumulated into meiotic asters and spindle, while the rest still distributed throughout the cytoplasm. These observations imply the occurrence of several distinct subcellular localization of MPF. To study further the interaction of MPF with microtubules, sucl-affinity purified starfish MPF was incubated with purified porcine brain microtubules, or microtubules were prepared from maturing starfish oocytes with the aid of Taxol. Western blots of microtubule precipitates probed with anti-cyclin B and anti-PSTAIR antibodies revealed that MPF associates directly with microtubules. Addition of ATP to MPF-associated microtubules caused the phosphorylation of several microtubule proteins, suggesting that MPF phosphorylates microtubule proteins to affect the instability of microtubules.