chain reaction and Northern blot analysis showed that the expression level of the gene developmentally increased. By screening the rat testis cDNA library, we successfully isolated rat cDNA clones encoding the entire open-reading frame of 1,671 base pairs coding 557 amino acids. In situ hybridization revealed that this gene was exclusively expressed in spermatids (step 7-17) in rat testis. We will also demonstrate the expression and the localization of this protein in rat testis by immunohistochemistry.

PROFILE ANALYSES OF SPERGEN-2 EXPRESSED IN RAT TESTIS SPERMATOGENIC CELLS

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By use of differential display in combination with cDNA cloning approach, we successfully isolated a novel gene termed spergen-2, which had an open reading frame of 1,500-length nucleotides encoding a protein of 500 amino acids. Spergen-2 contains ankyrin repeat motifs and a putative nuclear localization signal. We found that the expression of spergen-2 was developmentally up-regulated and that it was exclusively expressed in testis. In situ hybridization revealed that spergen-2 mRNA was expressed in spermatocytes and round spermatids. Immunohistochemical analysis demonstrated that spergen-2 protein was predominantly expressed in nuclei of late spermatocytes (stage IX~XIV) and spermatids (step 1~11), indicating the restricted expression of spergen-2 during spermatogenesis. In nucleoplasm of spermatogenic cell nuclei, spergen-2 tended to localize in the interchromosome space with relatively low density of DNA. Protein elution experiments using isolated spermatogenic cell nuclei revealed that spergen-2 protein was eluted from the nuclei by treatment of DNase I followed by exposure to 0.33 NaCl, suggesting that spergen-2 is a chromatin-associated protein.

ENDOCRINE CONTROL OF OOCYTE MATURATION AND SPAWNING IN BIVALVES

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In *in vitro* experiment using scallop ovarian tissue, serotonin (5-HT) induced oocyte maturation and subsequent egg release through the gonoduct in the presence of extracellular Ca^{2+}. Oocyte maturation appears to be the most critical event leading to egg released from the ovary. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) strongly inhibited 5-HT-induced egg release, but not oocyte maturation, thus suggesting that $PGF_{2\alpha}$ may suppress 5-HT-induced ciliary movement in the gonoduct. We found a novel factor from cerebral and pedal ganglia of the scallop to inhibit 5-HT-induced oocyte maturation and thereby result in the suppression of egg release. The novel oocyte maturation inhibitor (OMI) was characterized to be a 52 kD, heat labile protein. OMI showed the same function in various bivalves (e.g., *Chlamis farreri nipponensis*, *Crassostrea gigas*, *Spisula sachalinensis*, and *Ruditapes philippinarum*). OMI reduced an increase in intracellular Ca^{2+} induced by 5-HT, suggesting that the OMI inhibits an extracellular Ca^{2+} influx into oocyte *via* Ca^{2+} channels. It is thought that the spawning of bivalves triggered by 5-HT is regulated by $PGF_{2\alpha}$ and OMI at the different sites.

SIGANALLING PATHWAYS LEADING TO MEIOTIC MATURATION IN STARFISH OOCYTES

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Oocyte maturation in starfish, Asterina pectinifera, is induced by the natural hormone, 1-methyladenine (1-MeAde). We have previously reported that signalling pathways from 1-MeAde to activation of cyclin B/Cdc2 kinase are mediated by Akt/PKB which directly phosphorylates and downregulates Myt1 kinase in downstream of Gβ γ-PI3-kinase pathways. Here we found PKB/Akt also directly phosphorylates Cdc25 and upregulates Cdc25 activity in vitro. Thus, Akt/PKB acts as the trigger kinase which coordinately regulates both Myt1 and Cdc25 for the initial activation of cyclin B/Cdc2.

ANALYSIS OF MEIOTIC METAPHASE I ARREST IN THE HYMENOPTRAN INSECT, *ATHALIA ROSAE.*

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In vertebrate eggs the meiosis is arrested at the metaphase of meiosis II, and it resumes upon fertilization. The protooncogene *c-mos* product plays a role of the cytostatic factor. Mos activates the MAP kinase cascade. In contrast, in insect eggs, the meiosis is arrested at the metaphase of meiosis I. The regulatory mechanisms of meiotic arrest are still unknown in insects. We investigated events occurred during meiosis in insect using *Athalia rosae* in which unfertilized eggs can be easily activated *in vitro*. We first examined the MAP kinase activities upon egg activation. In unfertilized eggs, the MAP kinase was consistently activated. Once eggs were activated and meiosis was reinitiated, the MAP kinase was inactivated. The results suggested that the MAP kinase pathway partook in insect meiosis. Next, we examined whether an interspecific Mos acts as an upstream regulator of MAP kinase. The starfish Mos protein, the only Mos homologue obtained from invertebrate, was injected into *A. rosae* eggs. Many of Mos-injected eggs stopped development. For further examination of the Mos function, we established transgenic *A. rosae* strains that carry the starfish *c-mos* gene and its derivatives.

DIETHYLSTILBESTROL INDUCES FISH OOCYTE MATURATION

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Oocyte maturation in lower vertebrates is triggered by maturation-inducing hormone (MIH), which acts on receptors located on the oocyte membrane and induces the activation of maturation-promoting factor (MPF) in the oocyte cytoplasm. Here we show that exposing fish oocytes to DES at a dose within a range similar to that used in experimental exposure to natural MIH, 17a, 20b-dihydroxy-4-pregnen-3-one (Diol) induces oocyte maturation.An endocrine-disrupting chemical, DES, a nonsteroidal substance, triggered oocyte maturation in fish. The morphology (the time course of the change in germinal vesicle breakdown) and an intracellular molecular event (the de novo synthesis of cyclin B) induced by DES were indistinguishable from those induced by Diol. A synergistic action of DES on Diol-induced oocyte maturation was observed. These results suggest that DES acts on the MIH receptor as an agonist of Diol. The structural requirement for the action of DES was discussed based on results obtained using DES analogs.

IDENTIFICATION OF A PROTEIN KINASE WHICH PHOSPHORYLATES α4 SUBUNIT OF THE 26S PROTEASOME IN GOLDFISH OOCYTES

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The proteasome is involved in the progression of meiotic cell cycle in fish oocytes. We reported that the α4 subunit, which consists of 20S proteasome, is phosphorylated in immature oocytes and dephosphorylated in mature oocytes. To investigate the role of the phosphorylation, we purified the protein kinase from immature oocytes using recombinant α4 subunit as a substrate. Amino acid sequence analysis was performed for resulting partial purified fraction. A protein band which well corresponded to the kinase activity was identified as Casein Kinase Iα (CKIα). Phosphorylation of recombinant α4 subunit by the fraction was blocked by CKI-7, a specific inhibitor of CKI. In addition, we examined phosphorylation of α4 subunit which included in the 26S protesome complex. CKIα phosphorylated 26S proteasome from immature oocytes, but not phosphorylated that from mature oocytes. We further demonstrated that CKIα purified from mature oocytes possessed no activity for phosphorylation of α4 subunit. These results suggest that phosphorylation of α4 subunit is regulated by changes in the state of α4 subunit and activity of CKIα.

MOLECULAR CLONING OF MEDAKA MEMBRANE PROGESTIN RECEPTORS

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Fish maturation-inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), were shown to act *via* a heterotrimeric G-protein system to induce oocyte maturation. However, the detail of the action has been poorly understood. Recently, the cDNA of membrane progestin receptor was identified in spotted seatrout. Based on the sequence we have cloned two cDNAs from medaka ovarian cDNA library, OlGPCPR-α (*Oryzias latipes* G-protein-coupled progestin receptor-α) and OlGPCPR-β. The cDNAs of OlGPCPR-α and OlGPCPR-β are 2983 bp and 1566 bp in lengths, respectively. Each of the cDNAs has an open reading frame encoding a protein of 352 amino acids. Sequence analysis revealed that both OlGPCPR-α and OlGPCPR-β belonged to the G-protein-coupled receptor family with typical 7 transmembrane domains. The amino acid sequence of OlGPCPR-α has 86.9% identity with the seatrout sequence and 48.4% with OlGPCR-β. Northern blot analysis showed that OlGPCPR-α was expressed in brain, heart, kidney, intestine, ovary and testis, in contrast, OlGPCPR-β was expressed in ovary and testis. Rabbit polyclonal antibodies were generated for Western blotting analysis of the receptor proteins.