ISOLATION AND CHARACTERIZATION OF 51-KD ASTER FORMING PROTEIN FROM STARFISH OOCYTES.

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51-kD protein (p51) was isolated from starfish oocytes according to the method used for sea urchin egg p51, a component of microtubule-organizing granules in the mitotic apparatus. Starfish p51 showed similar properties to sea urchin egg p51. Starfish p51 was precipitated in a low salt solution and solubilized in 0.5M KCl, and reacted with monoclonal antibodies directed to sea urchin egg p51. Starfish p51 had an ability to form asters in vitro by incubation with porcine brain tubulin. Immunofluorescent staining revealed the localization of p51 at foci of cortical microtubules in immature oocytes and in the meiotic and mitotic spindles. p51, which could not form asters in vitro, was prepared from the low salt soluble fraction. Although the soluble p51 showed a similar peptide map to the precipitated p51, the soluble p51 fraction contained 42-kD protein phosphorylated by cdc2 kinase while the precipitated p51 fraction did not. Phosphorylation of 42-kD protein might regulate the aster forming ability of p51 during mitotic cycle.

INVOLVEMENT OF PROTEASOME IN OOCYTE MATURATION OF GOLDFISH (*Carassius auratus*)

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Proteasome activity was measured in oocytes from goldfish during 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP)-induced meiotic maturation. Enzyme assays were performed using a fluorogenic substrate (Suc-Leu-Leu-Val-Tyr-MCA) specific for proteasome. Oocyte proteasome activity fluctuated over the meiotic cycle with two peaks, one immediately prior to the migration of germinal vesicle(GVM) and the other just after the completion of germinal vasicle breakdown(GVBD);between these two peaks proteasome activity significantly decreased occuring before GVBD. Diisopropyl fluorophosphate(DFP), a serine protease inhibitor, prevented oocyte maturation. Experiments with one hour-pulse application of DFP revealed the existence of two DFP sensitive periods during 17α,20β-DP-induced oocyte maturation in goldfish. Oocytes treated with DFP during the first peak of proteasome activity did not undergo GVM. When oocytes with low proteasome activity were treated with DFP prior to GVBD, GVM was completed,but without GVBD. Using four chromatography columns, proteasome was purified from goldfish ovaries. Purified goldfish proteasome showed a single band on Native-PAGE and 8-9 bands on SDS-PAGE, indicating that goldfish proteasome is a multisubunit complex.

CHANGES OF CDC2 KINASE AND CYCLIN B DURING OOCYTE MATURATION IN FISH.

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We examined changes of protein levels of cdc2 kinase and cyclin B during goldfish oocyte maturation induced *in vitro* by 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), by using monoclonal antibodies against the PSTAIR sequence of cdc2 kinase and *E. coli*-produced goldfish cyclin B. cdc2 kinase was found in immature oocyte extracts and did not show any remarkable changes during oocyte maturation. Unlike cdc2 kinase, cyclin B was absent in immature oocyte extracts and began to be synthesized when oocytes underwent germinal vesicle breakdown. The synthesized cyclin B formed a complex with a preexisting cdc2 kinase, yielding an active MPF. Addition of *E. coli*-produced cyclin B into immature oocyte extracts induced MPF activation, which was associated with threonine phosphorylation of cdc2 kinase, as found in oocytes matured by 17α,20β-DP. Cyclin B-induced MPF activation was not induced by inhibiting threonine phosphorylation of cdc2 kinase. These results suggest that 17α,20β-DP induces immature oocytes to synthesize cyclin B, which in turn activates cdc2 kinase by inducing threonine phosphorylation of cdc2 kinase.

DISTRIBUTION OF PROTEASOME ANTIGEN IN EGGS DURING ASCIDIAN MEIOTIC DIVISION.

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Proteasome (multicatalytic proteinase) is a high-molecular weight protein complex composed of several components and shows several different protease activities.

We have previously reported that the proteasome undergoes a cell cycle-dependent change of its localization during cleavage cycle of fertilized eggs of the ascidian, Halocynthia roretzi.

In this study, we analyzed the distribution of proteasome during meiotic division cycle of H. roretzi eggs activated by the addition of calcium ionophore, A23187, immunocytochemically using monoclonal antibody raised against egg proteasome. The proteasome antigen appeared accumulated in the condensed chromosomes in the unfertilized eggs arrested at metaphase of the first meiotic division, and remained associated with the dividing chromosomes in the activated eggs during the anaphase. On the other hand, it was undetectable in the chromosomal region during polar body formation, but again detectable in the chromosomal region during the second meiotic division. These results suggest that the proteasome plays an essential role in the progression of meiotic division, especially from metaphase to anaphase.