PURIFICATION AND CHARACTERIZATION OF A CASEINOLYTIC PROTEASE IN HATCH WATER OF AN ESTUARINE CRAB, *SESARMA HAEMATOCHEIR*

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A caseinolytic protease is contained in crab hatch water (i.e. the filtered medium in which zoeas have hatched). It would digest the inner thin layer of the embryonic envelope to cause hatching. This protease has been purified from hatch water of an estuarine crab, Sesarma haematocheir, by three steps of chromatography. The molecular weight was estimated at 55 kDa by gel filtration. This protease was then characterized by use of highly purified solution; it was stable at 4-60℃ but was lost at higher temperature; the activity was maximum at pH 9.0-11.0; in prolonged incubation at 20℃ indicated that the activity was maintained at least for 72h. The assay with isolates of the embryonic envelope and morphological observation by the transmission electron microscopy suggested that the envelope was certainly digested by this protease.

OVIGEROUS-HAIR STRIPPING SUBSTANCE (OHSS) IN CRAB HATCH WATER: PURIFICATION, POLYCHRONAL ANTIBODY, AND THE SYNTHESIS OF OHSS IN EMBRYONIC DEVELOPMENT.

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Crab hatch water contains an active substance we call OHSS (ovigerous-hair stripping substance). This factor participates in cleaning the remnants that remained in the female's abdomen after the larval release, preparing incubation of the next clutch of embryos. Polychronal anti-OHSS antibodies were raised against purified fractions through gel filtration. This antibody detected 30KDa and 32KDa protein bands on SDS-PAGE. Affinity purification indicated that OHSS is composed of these two forms. Furthermore, synthesis of OHSS in embryonic development has been investigated by use of these antibodies.

Formation of embryonic envelope and the embryo attachment system in an estuarine crab.

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In decapod crustaceans, newly-extruded eggs are attached to maternal ovigerous hairs by a stalk(funiculus) until hatching occurs. The funiculus is not connected to the hairs directly, but indirectly through the "coat" investing ovigerous hairs. Formative process of funiculus and investment coat was investigated at ultrastructural level. Following egg laying, the structure of the embryonic envelope (E1) was rapidly modified, and envelope E1 adhered to the hair with many minute protrusions longitudinally arranged on the ovigerous hairs. Envelope E1 was then streched by vigorous keading movements of maternal pleopods, coilling around the hairs, which made up the coat and funiculus. Our studies thus suggest that the attachment system is all formed by the vitelline layer originated from eggs.

MECHANISMS OF PARTHENOGENETIC ACTIVATION OF C-*MOS*-DEFICIENT MOUSE OOCYTES

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Gene targeting experiments have shown that c-*mos* proto-oncogene product (Mos) plays an essential role in mouse oocyte maturation. However, mechanisms by which Mos suppresses the parthenogenetic activation of the mouse oocytes arrested at the second meiotic metaphase remain to be elucidated. Here we analyzed the protein levels of cyclin B and CDC2 kinase during the activation of the c-*mos*-deficient oocytes. CDC2 levels remain to be constant during maturation of both c-*mos*-deficient and wild type oocytes. In contrast, cyclin B was accumulated during second meiosis much slower in c-*mos*-deficient oocytes than the wild-type ones. Furthermore, metabolic labeling experiments suggest that Mos kinase functions as a positive regulator of cyclin B protein synthesis.

PHOSPHORYLATION STATE OF CDC2 ON THREONINE161(T161) DURING MOUSE OOCYTE MATURATION.

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CDC2 kinase a general regulator of eukaryotic cell cycle, plays a key role in the regulation of oocyte maturation.Activation of CDC2 kinase is depend on both the association with cyclinB and the phosphorylation on threonine 161(T161). In this study, we prepared antibody against CDC2 phosphorylated on T161 and analyzed the phosphorylation state of CDC2 during mouse oocyte maturation. T161-phosphorylation levels were low in the immature and activated oocytes,where CDC2 kinase was inactivated. In contrast, T161-phosphorylation levels didn't decrease upon the first polar body emission even though the activity of CDC2 kinase was declined to the trace levels.

CHANGES OF DESMIN EXPRESSION RELATED TO MUSCLE REMODELING DURING METAMORPHOSIS OF IN *XENOPUS LAEVIS*

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During metamorphosis of the frog *Xenopus laevis*, both the death of larval type muscle cells and new myogenesis occur to form adult dorsal muscles. The new myogenesis area expand in a wave-like fashion with an anteroposterior gradient during metamorphic climax stage. The muscle cell death area expanded posterior-anteriorly.

In order to know the mechanism of muscle remodeling from larval type to adult one during metamorphosis, changes in protein profile of dorsal and tail muscles of *Xenopus laevis* was examined. The content of desmin, muscle intermediate filament protein, per muscle total protein was found to decrease dramatically during metamorphosis. Before the desmin decrease, intense desmin expression near the adult type myogenesis area (a dorsomedial part in muscle cross section) was observed. Detailed western blot analysis showed that desmin decrease also proceeded with axial gradient. These data suggested the importance of the desmin change in muscle remodeling during metamorphosis.