It is noteworthy that 25R-SAAF (SAAF epimer) possess the same two activities as 25S-SAAF. Thus, epimerism at C-25 is no effect on the SAAF activities.

THE ACROSOME REACTION-INDUCING SUBSTANCE ON THE VITELLINE ENVELOPE IN *XENOPUS LAEVIS*

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Although the acrosome reaction (AR) in sperm is indispensable for fertilization of many animals, its molecular mechanism remains to be investigated in amphibians. To identify an acrosome reaction-inducing substance in *Xenopus* (ARISX), a monoclonal antibody against ARISX was raised by immunization with a pars recta extract (PRE) containing ARISX. The anti-ARISX antibody which recognizes the carbohydrate moiety inhibited the AR induced by PRE or on the VE in a dose-dependent manner. The ARISX in PRE was lost the AR-inducing activity by periodate oxidation. The treatment of PRE with several lectins precipitated ARISX but SBA and DBA inhibited the activity of ARISX. The activity of ARISX was not inhibited by the treatment with several proteases. These results indicate that terminal GalNAc residues in ARISX are impotant for the acrosome reaction-inducing activity.

SEA URCHIN SPERM FLAGELLA CONTAINS GLYCOPROTEIN WITH α2→9-LINKED POLYSIALIC ACID CHAINS

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Previously, we showed enrichment of a sulfated ganglioside HSO_3→8Neu5Acα2→8Neu5Acα2→6GlcCer in membrane microdomains of sea urchin sperm. We also found that the HSO_3→8Neu5Ac structure was expressed on certain glycoproteins of sea urchin sperm. To elucidate the structure of HSO_3→8Neu5Ac-containing glycans, the major sialic acid-containing glycopeptide fraction (designated SGP) was prepared from sea urchin sperm by exhaustive Actinase E digestion. The structure of glycan chains of SGP was determined by various chemical analyses and established as follows: HSO_3→8Neu5Acα2→9(Neu5Acα2→9)$_{n-2}$Neu5Acα2→6GalNAc1→Ser/Thr (n = about 15). This novel polysialic acid chain was shown to be localized on sea urchin sperm flagella. This suggests the involvement in sperm activation or sperm motility at early stages of fertilization rather than in acrosome reaction or sperm-egg fusion. This is the first evidence for the occurrence of polysialic acids in animal sperm.

THE APOPTOTIC SIGNAL FROM SEA URCHIN SPERM DURING FERTILIZATION

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We studied the interaction between egg and sperm of sea urchin, *Anthocidaris crassispina*. After treatment on ice more than three hours, the unfertilized eggs lost the capacity of fertilization, but were able to bind the sperm. This new egg model, "cooled egg", is available to observe adhesion of sperm to egg surface using fluorescent dyes. We used as a fluorescent probe annexin V-FITC which reacted with phosphatidyl serine translocated to the cell surface in the early stages of apoptosis. Some of the sperm adhered to the surface of the cooled egg showed the fluorescence of annexin V-FITC. The cooled egg or sperm alone did not show any signal of the fluorescence. Presence of jelly around cooled egg did not affect the fluorescence of the sperm. We discuss the meaning of the apoptotic signals of the sperm during fertilization.

ANALYSIS OF TCBP-25 (*TETRAHYMENA* CA^{2+}-BINDING PROTEIN OF 25 kDa) DURING SEXUAL REPRODUCTION IN *TETRAHYMENA THERMOPHILA*

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TCBP-25 is localized around both the migratory and stationary gametic pronuclei at the pronuclear exchange stage during conjugation. The function of TCBP-25 during sexual reproduction was not elucidated. To understand the function of TCBP-25, we analyzed the localization of TCBP-25 in conjugation mutants and executed the reduction in gene expression of TCBP-25, and then analyzed the phenotypic effects. According to the localization of this protein and its timing, three possible roles of TCBP-25 are proposed. TCBP-25 plays roles in 1) the distinction between the two functional gametic pronuclei and the other three degenerative nuclei, 2) the migratory pronuclear exchange and 3) the pronuclear fusion. To verify these hypotheses, the localization of TCBP-25 in conjugation mutants (*cnj10, cnj7* and *bcd2*) was examined. The results suggest that TCBP-25 should play a role in the migratory pronuclear exchange. We succeeded in the reduction in gene expression of TCBP-25 using the antisense ribosome system, and analyzed the phenotype of the transformants. The knock down of TCBP-25 function equally suggests that TCBP-25 takes part in the migratory pronuclear exchange.

MULTIPLE FORMS OF CATHEPSIN L IN *HEMICENTROTUS PULCHERRIMUS*

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Previously we purified cathepsin L-like proteinase in the egg of sea urchin, *Hemicentrotus pulcherrimus*, as a MYP-degrading enzyme. As the participation of other proteinases was suggested, developmental changes of proteinase activity and zymographic pattern were examined. Cathepsin L-like activity was decreased after fertilization and increased again at and after MBT. Zymography showed that 32kDa activity was dominantly contained in the unfertilized eggs. Interestingly 40-42kDa activity was transiently increased at blastula stage and ovary extract dominantly expressed about 50-52kDa activity. These results indicated the presence of multiple forms of cysteine proteinases and they were differently used depending on tissues and developmental stages. Therefore, we investigated the mRNAs encoding cathepsin L-like proteinases in the total RNA of ovary of H. pulcherrimus, by RT-PCR with degenerate primer sets. Partial DNA sequence analyses revealed the presence of five different kinds of cathepsin L cDNA fragments. His residue in the active site and all 4 Cys residues in the fragments were conserved. These cDNAs are homologous to that of human pro-cathepsin L.

AN INHIBITOR FOR H^+/K^+-ATPase DISRUPTED THE LEFT-RIGHT ASYMMETRY IN THE ECHINOID EMBRYO

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The molecular basis in establishment of left-right asymmetries has been studied mainly invertebrate development. In chick and frog, it was reported that specification of left-right asymmetry is initiated by a differential ion flux created by H^+/K^+-ATPase activity in very early developmental stages. We treated embryos of the sea urchin *Hemicentrotus pulcherrimus* with an inhibitor of H^+/K^+-ATPase, and found that the sided pattern of an asymmetrically expressed gene was disrupted. Our results suggest that H^+/K^+-ATPase has a role in establishment of LR patterning in echinoid embryos.

A SEA URCHIN *HEMICENTROTUS PULCHERRIMUS* HOMOLOGUE *HpSu(H)* IS INVOLVED IN THE DIFFERENTIATION OF SECONDARY MESENCHYME CELLS

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Suppressor of hairless (Su(H)) is a key component of the Notch signalling. It is a transcription factor that acts as a repressor in the absence of Notch signal. If Notch signal is activated, it binds the released intracellular domain of Notch and acts as an activator of transcription. Notch signal mediates communication between adjacent cells and is employed for binary fate decisions. In order to examine the role of Notch signalling in sea urchin development, first we identified the sea urchin *Hemicentrotus pulcherrimus* homologue of Su(H). *HpSu(H)* mRNA exists from unfertilized egg to hatched blastula stage, then increases significantly after mesenchyme blastula stage. To understand the function of HpSu(H) we designed the experiment to perturb the embryo by inducing ectopic overexpression of dominant negative form Su(H), which does not bind DNA but interacts with Notch, by injecting mRNA to fertilized eggs. The overexpression of dominant negative Su(H) specifically repressed the differentiation of secondary mesenchyme cells (SMCs) such as pigment cells and blastocoelar cells. This result suggests that Su(H) is involved in the differentiation of SMCs in sea urchin development.