ROLES OF ACTIN NETWORKS IN PERISTALTIC SQUEEZING DURING SPERM MATURATION IN *BOMBYX MORI*

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Two types of sperm are produced in the silkworm, *Bombyx mori*. Nucleate eupyrene sperm is an ordinary sperm that contributes to fertilization, while anucleate apyrene sperm is considered to play important roles in assisting eupyrene sperm. At the very late stage of spermatogenesis, a phenomenon called 'peristaltic squeezing' occurs in both types of sperm, whereby cytoplasm of the eupyrene and nuclei of the apyrene sperm are discarded from the posterior end, forming matured sperm. The squeezing action is inhibited by addition of Cytochalasin D, inhibitor for motility of actin filaments, in the culture medium. In this study, rhodamine-phalloidin staining for actin was applied to sperm bundles, and confirmed that actin filament networks spread on the cyst cells, as well as actin particles within the bundle, are responsible for the squeezing action.

INFLUENCE OF HIGH TEMPERATURE TREATMENT ON DIMORPHIC SPERMATOGENESIS IN *BOMBYX*

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Silkworm males produce dimorphic sperm, nucleate eupyrene and anucleate apyrene sperm. Meiosis in the two types of sperm occurs definitely different periods of development; eupyrene spermatocytes start meiosis just after the last larval molting, while apyrene spermatocytes after the spinning stage. When males of Daizo (one of the silkworm strains) at the spinning stage were placed in a high temperature (33°C) condition for 96h, they became sterile. Eupyrene spermatogenesis to matured sperm proceeded normally, whereas abnormality appeared only in apyrene spermatogenesis. Extension of flagellar axoneme and the unidirectional orientation of sperm in a cyst were disturbed. Furthermore, peristaltic squeezing from the anterior to posterior end was unsuccessful. Submicroscopic observations disclosed collapses of 9+2 structures of the axonemes. Such disturbance in axoneme structure was not seen in both sperm types when the high temperature treatment was applied during the final larval stage. These results showed that abnormality of spermatogenesis in the silkworm was induced by the high temperature treatment during the stage when the apyrene meiosis was proceeding.

NEURAL NETWORKS IN OVARIES AND TESTES OF THE STARFISH *ASTERINA PECTINIFERA*

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The nervous system plays important roles in oogenesis and spermatogenesis in both vertebrates and invertebrates. In starfish, the peptide hormone GSS secreted from nervous tissue stimulates oocyte maturation to induce 1-methyladenine production by ovarian follicle cells. We have investigated the organization of the neural networks in ovaries and testes of the starfish *Asterina pectinifera* by employing an antibody that recognizes molecules belonging to the SALMFamide family of echinoderm neuropeptides. Following incubtion with a SALMFamide 1 primary antibody, followed by either an Alexa Fluor- or rhodamine-conjugated second antibody preparations were analyzed using a confocal LASER scanning microscope. Results revealed neural networks in both ovaries and testes. The networks were restricted mainly to the surface of both ovaries and testes and little evidence of immunoreactivity was seen inside their basement membranes. In constrast, use of antibodies against serotonin revealed patch-like areas of immunoreactivities surrounding the oocyte. These results suggest that neural networks distributed in gonads may be involved in the regulation of gametogenesis in starfish.

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST 530kDa PROTEASOME-ASSOCIABLE COMPLEX (PC530) FROM STARFISH OOCYTES

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We have previously reported that the oocyte proteasome undergoes changes in activity under the influence of 1-methyladenine before GVBD and that the proteasome plays an important role in oocyte maturation. We also reported the characterization of a 530-kDa 20S proteasome-associable complex (PC530) purified from oocytes of the starfish, *Asterina pectinifera* (Tanaka *et al.*, 2000). Although we revealed that this complex is able to associate with the 20S proteasome *in vitro*, the physiological functions of PC530 have not been elucidated. In order to know the roles of PC530 in starfish oocyte maturation, we made several monoclonal antibodies in mice against the *A. pectinifera* PC530. Among several antibodies, a monoclonal antibody 7C5 is able to delay the maturation process of starfish oocyte, which is elicited by 1-methyladenine treatment. These results indicate that the PC530 plays a key role in the oocyte maturation process in *A. pectinifera*. (REFERENCE: Tanaka E, Takagi Sawada, M, Morinaga C, Yokosawa H, Sawada H (2000) Arch Biochem Biophys 374:181-188)

PHOSPHORYLATION OF STARFISH STEM-LOOP BINDING PROTEIN IN STARFISH OOCYTE EXTRACT

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Histone genes lack introns and the mature histone mRNAs terminate with a stem-loop secondary structure rather than poly A tails. The stem-loop interacts with a specific RNA-binding protein, termed the stem-loop binding protein (SLBP) or hairpin binding protein (HBP), which participates in pre-mRNA processing and remains associated with the mature histone mRNA. The stem-loop is also essential for translation, presumably as a complex with SLBP. Fully grown, immature starfish oocytes are arrested at the G2/M-phase border of meiosis I. Maturation is induced by a hormone, 1-methyladenine (1-MA). The signal of 1-MA stimulates the heterotrimeric G protein, resulting in dissociation of the βγ subunit of G protein (Gβγ). To investigate the targets for Gβγ, we analyzed Gβγ-dependent phosphorylation of proteins by screening the expression cDNA library. Then we cloned the gene encoded a protein containing a region identical to RNA binding domain of SLBP. Here, we report that the starfish protein binds to the stem-loop of starfish histone mRNA.

DETECTION OF UNIQUE DNA LOCALIZED IN THE STARFISH SPERM CENTROSOME FROM PARTHENOGENETICALLY ACTIVATED EMBRYOS

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In most animals, only the sperm centrosome provides the division poles for mitosis in zygotes. We found novel DNA from the sperm centrosome of the starfish *Asterina pectinifera*. About 1 kb of the partial sequence of the novel DNA was identified. The sequence is different from that of the mitochondrial DNA of this species. In this study, in order to understand the fate of this DNA in sperm centrosome and to identify the localization of the novel DNA in the embryo, normally fertilized embryos and parthenogenetically activated embryos of starfish were investigated. The total DNA of both embryos was isolated respectively to PCR. Using the newly designed primers that were based on the novel DNA sequence, PCR analysis was performed. The DNA sequence of PCR products completely corresponded with the novel DNA of sperm centrosomes. Using the whole-mount *in situ* PCR, the localization of the DNA in these embryos was investigated. These signals were confirmed from the basal bodies of cilia that are localized on the surface of the embryo. These results indicated the presence of a unique DNA in the centrosome of the starfish sperm and embryos.

INTRACELLULAR pH INCREASE AT MEIOSIS REINITIATION IN STARFISH OOCYTES

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Fully grown starfish oocytes are arrested at prophase of meiosis I. Meiosis is reinitiated by the hormonal stimulation of 1-methyladenine (1-MA), which dissociates G protein βγ subunit from G protein α subunit. The dissociated G protein βγ subunit (G βγ) activates PI3 kinase (PI3K), which results in the formation of MPF, inducing germinal vesicle breakdown (GVBD). The Na^+/H^+ antiporters are known to regulate intracellular pH (pH_i) in various cells. They are plasma membrane proteins catalyzing the electroneutral exchange of intracellular H^+ for extracellular Na^+. We have demonstrated that Na^+/H^+ antiporters are involved in a 1-MA-induced pH_i increase, and injection of G βγ into oocytes induces the pH_i increase without 1-MA stimulation. Also PI3K inhibitor (LY294002) block 1-MA-dependent pH_i increase, indicating that Na^+/H^+ antiporters are regulated by PI3K. In this study, we found that inhibition of pH_i increase by using Na^+ free seawater blocked polar body formation of 1-MA-treated oocytes, indicating that pH_i increase is prerequisite to polar body formation.

REPRODUCTIVE CAPACITY OF THE CENTROSOME IN STARFISH IMMATURE OOCYTES

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Immature oocytes of the starfish, *Asterina pectinifera*, were deprived of the germinal vesicle and were fused with a mature egg. Formed conjugates were then activated and observed for further development. We found two asters which came out following pronuclear breakdown. Number of the forming asters was always two.