egg membranes. When unfertilized eggs were treated with 1mM 2-APB, an inhibitor of IP3 receptors, the egg-activation was completely inhibited. The 2-APB treatment inhibited a positive fertilization potential, but did not inhibit small spikes. These results indicate that Ca^{2+} ions are necessary for the process of sperm-egg membrane contact and/or fusion at fertilization.

INDUCTION OF SPERM ACROSOME REACTION BY EGG ENVELOP GLYCOPROTEIN ZP1 AND ZPC OF JAPANESE QUAIL (*COTURNIX JAPONICA*)

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The extracellular matrix surrounding avian oocytes, termed as perivitelline membrane (PL), is consisted of at least two major glycoproteins. Our previous studies of Japanese quail have demonstrated that one of its components, ZPC, is synthesized in the ovarian granulosa cell, whereas another component, ZP1, is synthesized in the liver. However, little is known about the role of these glycoproteins in fertilization. In order to understand the process of fertilization in bird, we evaluated the effects of ZP1 and ZPC on the induction of sperm acrosome reaction in Japanese quail. Spermatozoa were incubated with or without the purified glycoprotein. After the incubation, acrosome status was observed based on the presence or absence of the acrosome. Treatment of spermatozoa with increasing dose of the purified dimeric ZP1 led to a concentration-dependent stimulation of acrosome reaction. The monomeric ZP1 had similar effect. Moreover, we found that the ZP1-induced acrosome reaction was significantly blocked by the pretreatment of the purified ZP1 with PNGaseF. On the other hand, the addition of the purified ZPC failed to induce acrosome reaction at any doses tested.

ULTRASTRUCTURAL CHANGE OF THE TREE-FROG SPERMATOZOA IN THE FOAM NEST

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Fertilization of *Rhacophorus arboreus* ocurr in the foam nest. The spiral-shaped spermatozoon has a flagellum with a pair of axonemes surrounded by many microtubules (Hongo and Matsui, 2004). To investigate the sperm morphology in the foam nest, we observed them with light and electron microscpes with time passages.Within a half day after egg laying, no spermatozoa moved in the nest which consisted of fibrous material with many lipid droples and vacuoles. After tadpoles appeared, the sperm shape were changed to be a elongated coil.

Cdk(CYCLIN-DEPENDENT KINASE) IS REQUIRED FOR GENOMIC UNION IN THE FERTILIZED STARFISH EGGS

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Sexual reproduction requires both meiosis to produce haploid gamates and fertilization to unite the gamates. Although the process of fertilization is different in different animals depend on the timing of sperm penetration in the meiosis, in most animals fertilization requires the contact of the egg and sperm and their migration on microtubules, and is complete the parental genomes unite. Neither the mechanism of the migration of male and female pronuclei nor the mechanism to unite them has been fully understood. Cyclin-dependent kinases (Cdks) are known as regulators involved in pivotal events in cell cycle. Cdk is also involved in membrane traffic and fusion. In the present study we examined whether Cdk plays a role in genomic union using an inhibitor of Cdks, GW8510 and cyclin antisense oligonucleotides. In the fertilized eggs treated with GW8510 and injected with cyclin A antisese oligonucleotide the female and male pronuclei migrated close to each other but they did not fuse. These results show that Cdks is required for the union of the male and female genomes in starfish fertilized eggs.

CLONING OF A MEDAKA HOMOLOGUE TO *PHOSPHOLIPASE C* ZETA GENE THAT ACTS AS SPERM FACTOR IN MAMMALIAN FERTILIZATION

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At the time of fertilization, egg is activated by rise of intercellular Ca^{2+} concentration. In mammals, phospholipase C zeta (PLCZ) is reported to play a critical role as sperm factor, which induces Ca^{2+} oscillation in fertilized egg. On the other hand, the role of PLCZ in the other vertebrates is remained unknown. Medaka (*Oryzias latipes*) has been used as an important experimental animal for a long time. Recently, medaka genomic resources, such as genome and EST sequences, are rapidly being accumulated. To understand the role of PLCZ in fish, we performed *in silico* cloning and identified medaka *PLCZ* gene. We found genome regions in medaka and fugu genomes, which contain sequence homologous to mammalian *PLCZ* gene. Clustal analysis revealed that the coded PLC does not belong to PLC delta groups, but belongs to the PLCZ group, strongly suggesting that the *PLC* gene encodes medaka PLCZ. The deduced medaka PLCZ protein (567 amino acid residues) contains 2 EF hand like-domains, and X, Y, and C2 domains. Medaka *PLCZ* is expressed strongly in the testis but not in other organs examined, suggesting that PLCZ has a significant role in medaka fertilization.

THE DEGRADATION OF SEMENOGELINS BY HUMAN SPERM

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During liquefaction of human semen, semenogelin-I and -II (Sgs), which are gel-forming proteins and secreted from seminal vesicle are degradated by prostate specific antigen (PSA), resulted in sperm motility activation. Sgs are degradated by PSA to generate peptides of various biological activities. One is a 14-kDa protein, seminal plasma motility inhibitor believed to have an inhibitory effect on the ability of sperm to move. The other is unknown degradation product of Sgs have an inhibitory effect on capacitation by interfering with the superoxiside anion generated during this process. The physiological substrates of PSA are Sgs, however the process of further modification on the degradation products is still unclear. In this report, we will show the process about degradation of Sgs by 2D electrophoresis of the peptides degradated by PSA or human sperm.

FERTILITY OF THE MOUSE SPERM WAS CONTROLLED BY SEMENOCLOTHIN, THE MAJOR PROTEIN OF SEMINAL VESICLE

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Seminal plasma contains substances or factors that control sperm fertility. The capacitated rabbit sperm reversibly lost their ability to fertilize when they were exposed to the seminal plasma. This phenomenon was called decapacitation. However, the factor inducing decapacitation and the mechanism are still unclear. In this study, we examined whether major protein of seminal vesicle, Semenoclothin (Sc) affects mouse sperm fertility *in vitro*. First, we investigated fertility of mouse sperm, capacitation, and acrosome reaction (AR). The sperm treated with 15mM HCO_3^-, 100 μM forskolin, and 100 μM M β CD did not show AR, but the addition of 10 μM ionomycin to these sperm promoted AR. This phenomenon was considered to be capacitation. On the other hand, the sperm treated with 100É M progesterone increased in AR without the addition of ionomycin. We regarded it as AR. Treatment of seminal vesicle fluid inhibited both sperm capacitation and AR. Moreover, the recombinant Sc also prevented sperm from occurring capacitation and AR. These results suggest that Sc plays a role in the decapacitation factor of mice.

ROLES OF HISTONE CHAPERONES FOR DNA REPLICATION-COUPLED NUCLEOSOME ASSEMBLY IN *XENOPUS* EARLY EMBRYOS

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In early embryonic cells, DNA replication and chromatin assembly should be particularly well coordinated because the replication of not only DNA but chromatin has to be finished within an S phase, which is as short as 15 min in *Xenopus* early embryos. Using *Xenopus* egg extracts, we have investigated roles of the histone chaperones Asf1 and CAF-1 in nucleosome assembly coupled to DNA replication in S phase. The results of immunoblot analysis and histological immunostaining show that Asf1 forms complexes with other histone chaperones nucleoplasmin, N1 and NAP-1 in both M and S phases and binds to CAF-1 only in S phase. They also show that both Asf1 and CAF-1 bind to chromatin while DNA replication is ongoing. We demonstrate through immunodepletion experiments that both histone chaperones are essential for replicated DNA to be assembled into regularly spaced nucleosomes in S phase and that the chaperone-dependent construction of the chromatin basic structure is required for the full extent of chromosome condensation in the subsequent M phase. The respective roles of Asf1 and CAF-1 in replication-coupled nucleosome assembly will be discussed.

CHANGES IN INTRACELLULAR CA^{2+} CONCENTRATION DURING EARLY EMBRYONIC CELL CYCLES IN *XENOPUS*

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Rapid and synchronous cell divisions are characteristics of the cell cycles during cleavage stages in the embryos of many animal species. The mechanism of regulation in cell cycle length remains to be investigated in vertebrates including amphibians. It has been reported that the intracellular concentration of Ca^{2+} ions ($[Ca^{2+}]_i$) changes or oscillates during cell cycles of cleavage, but its role in the regulation on cell cycle is still unclear. Since the frog blastomeres are opaque, the changes of $[Ca^{2+}]_i$ only in the cortex was able to measure in the previous studies. In this study, we measured the changes in $[Ca^{2+}]$ not only in the cytoplasm, but also in the nucleus during cleavage in the embryos of the frog, Xenopus laevis with the translucent blastomere system. The nuclear $[Ca^{2+}]$ increased during interphase, and suddenly decreased just after NEBD at the beginning of