CYTOPLASM OF STARFISH OOCYTES EXHIBITS CYCLIC CHANGES IN RIGIDITY INDEPENDENTLY OF THE GERMINAL VESICLE (GV) CONTENTS

K. Yamamoto[1], M. T. Seto[2], T. Yamamoto[2] and S. Nemoto[2].
[1]Dep. of Biol. Divers. Resour., Fac. of Agricul., Gifu Univ., Gifu.
[2]Dep. of Biol., Fac. of Sci., Ochanomizu Univ., Tokyo.

We have reported that "cytoplasmic cycle" (as indicated by cyclic changes in rigidity) is established under the influence of GV factor(s) in meiotic cell cycle of starfish oocytes. To know whether the cytoplasmic cycle in later phases (mitotic cell cycle) is also dependent on GV contents, the changes in rigidity of 1) the oocytes from which GV contents was sucked out by micropipet, or 2) bisected oocyte fragments which lack a GV were examined using *Asterina pectinifera*. They were induced to mature and activated with either sperm or Ca-ionophore. The rigidity was continuously monitored using a compression method.

We found that regardless of the method of enucleation or activation, oocyte cytoplasm exhibit remarkable cyclic changes in both rigidity and surface appearance which start 6-10 hr after 1-methyladenine application. The intervals of these cycles were 70-90 min (23℃) .

These results indicate that in starfish oocytes, a cytoplasmic cycle can be established independently of GV contents.

HETEROPLASMIC CONJUGATES FORMED BY FUSION OF STARFISH OOCYTE PAIRS WITH A 12-MINUTE TIME DIFFERENCE OF MATURATION PHASE

M. Yoneda. Takiyama 5-7-7, Higashikurume, Tokyo

Oocyte pairs of Asterina pectinifera with a 12 minute time difference of maturation phase were electrically fused together (Yoneda '97 for the method) to make heteroplasmic conjugates of the oocytes. Under polarization microscopy the two metaphase I spindles in a conjugate were found to form with a time difference of 9 to 12 minutes between each other. To the contrary Nomarski optics revealed the two first polar bodies forming concomitantly within 1 minute.
Based on these observations I conclude that there exists a certain factor in oocyte cytoplasm which triggers the exit from metaphase or anaphase of the first meiotic division in starfish oocyte.
A whole work was done at Tateyama Marine Laboratory, Ochanomizu University, Tateyama.

M. Yoneda(1997) Develop. Growth & Differ. 39:741-749

EXPRESSION OF THE SF-1/Ad4BP GENE IN GONADS AND BRAINS OF THE FROG, *Rana rugosa*.

K. Kawano, M. Takase and M. Nakamura.
Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Higashi-Hiroshima.

SF-1/Ad4BP is essential for transcriptional regulation of genes affecting adrenal and gonadal development and sex differentiation in mammals. It is also known to have a key role for regulation of steroidogenesis in gonads and brains in mammals. In order to clarify the role of SF-1/Ad4BP gene in frog gonads and brains, we cloned the full-length of SF-1/Ad4BP cDNA of the Japanese wrinkled frog (*Rana rugosa*) and sequenced. Using an antisense RNA probe, we analyzed its expression by the *in situ* hybridization method. SF-1/Ad4BP mRNA was found to be expressed in brains and testes, but not ovaries, of adult frogs. The results suggest that the SF-1/Ad4BP gene probably play an important role in steroidogenesis in frog brains and testes.

HISTOLOGICAL ANALYSES OF *FTZ-F1* GENE EXPRESSION IN AMPHIBIAN GONADS.

M. Takase, T. Nakajima and M. Nakamura
Lab. for Amphibian Biol., Fac. of Sci., Hiroshima Univ., Higashihiroshima.

To clarify the functions of the *FTZ-F1* gene in amphibian gonads, we investigated histologically its expression using the wrinkled frog *Rana rugosa*, after metamorphosis. Firstly, we examined the expression of FTZ-F1 protein in *Rana* testes and ovaries by immunohistochemical technique using rabbit IgG raised against FTZ-F1 polypeptides. The antibody detected a specific cell type in the interstitial space of the testis, and oocytes at early developmental stages in the ovary. In addition, localizations of FTZ-F1 mRNA in these cells were verified by *in situ* hybridization method using the antisense RNA probe. We further analyzed FTZ-F1 localizations in developing gonads. Based on these results, the function of the *FTZ-F1* gene is discussed.

ULTRASTRUCTURAL STUDIES ON THE BEHAVIOR OF CENTRIOLES DURING MEIOSIS OF SEA URCHIN OOCYTES.

S.Nakashima and K.H.Kato. Inst. of Natural Sci. Nagoya City Univ., Nagoya

There have been many ultrastructural studies on oogenesis in sea urchins. Little is known about the ultrastrucutural changes associated with meiotic events in sea urchins. Particularly interesting is the behavior of centrioles during meiosis.

We investigated the process of maturation of the sea urchins Hemicentrotus pulcherrimus and Temnopleurus hardwicki. Observations were focuses on the number and behavior of centrioles during two successive meiotic divisions. Examination of serial sections revealed that after the first meiotic division the oocyte has a pair of centrioles, and in the second meiosis each division pole has only one centriole, confirming the observation by Sluder et al. (1989) and Kato et al. (1990) on the oocytes of starfishes. The first polar body which was larger than the second one, had two centrioles and the second polar body had only one. Two centrioles in the first polar body did not separate after the first meiotic division. These results indicate no duplication of centrioles occurs during the two successive meiotic division, and that the egg inherits one centriole from a primary oocyte.

A HOMEOBOX GENE ISOLATED FROM A STALKED CRINOID, *METACRINUS ROTUNDUS*

T. Hibino[1], Y. Taniguchi[2], T. Higashinakagawa[3] and S. Amemiya[1]
([1]Dept. of Biol. Sci., Grad. Sch. of Sci., Univ. of Tokyo, [2]Div. of Mol. Life Sci., Tokai Univ. Sch. of Med., [3]Dept. of Biol., Sch. of Educ., Waseda Univ.)

Stalked crinoids are the most primitive group in Phylum Echinodermata. Phylogenetic analysis of the stalked crinoids has not yet been performed moleculaly. We tried to clone homeobox genes in a stalked crinoid, *Metacrinus rotundus*, in order to investigate the phylogenetic situation of the stalked crinoid among and within the Phylum. A homeobox gene was cloned from a genomic library of the spices by using a fragment of the consensus region in homeobox as a probe. The comparative analysis of a homeodomain with highly conserved protein motif of 60 amino acids in homeobox gene showed that the crinoid homeobox gene had the highest homology with two genes, Nkx-5.2 of mice and spHmx of sea urchins, belonging to H6 subfamily. However the rate of homology in the homeodomains between the crinoid homeobox gene and Nkx-5.2 or spHmx were less than 60%, suggesting that the gene belongs to a novel homeobox family.