ESTABLISHMENT A METHOD TO ANALYZE CELL CYCLE AND CELL DIFFERENTIATION UPON EARLY *XENOPUS* DEVELOPMENT USING TRANSPARENT BLASTOMERES

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The dissociated blastomeres of the frog, *Xenopus lavis* are available not only for the analysis of the cell cycle under a lower Ca concentration, but also for the analysis of cell differentiation in early development under a higher Ca concentration in culture medium. The blastomeres, however, are too opaque to observe the events occurring in inner cytoplasm of the blastomeres. In this study, we have developed a new procedure to make the blastomeres transparent by dissociating the blastomeres from the embryos that had been centrifuged for stratification of the cytoplasm. To analyze the duration of each cell cycle phase on real time, we next tried to visualize EGFP-PCNA in living transparent blastomeres. As the result, EGFP-PCNA diffused throughout cytoplasm during M phase, and next assembled in some small points, as like karyomeres, and more gathered during S phase. This result consists with previous report using fixed and immuno-stained embryos. In addition, for establishment of a system to analyze cell differentiation using the transparent blastomeres, we have determined a culture condition for the blastomeres to differentiate into several types of ectodermal tissue.

DOES WNT/β-CATENIN PATHWAY CONTROL THE STARFISH ARCHENTERON FORMATION THROUGH BRACHYURY?

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To completely elucidate the archenteron formation mechanism of starfish and to utilize this animal model to monitor the ocean contamination, we are analyzing the down-stream factors of Wnt/β-catenin pathway. It is well known that brachyury is a target gene of this pathway. Shoguchi et al (1999, 2000) supposed that *T-brain-1* homologue regulates the starfish archenteron formation. Last year, we reported that synthetic mRNA for the cadherin cytoplasmic domain strongly inhibited the archenteron formation when injected into starfish larvae. Furthermore, the amount of β-catenin in LiCl-treated blastulae was shown to be higher than normal larvae by western blotting analysis in our recent experiment. This study is aimed to measure the amounts of mRNA for two *T*-genes in animalized or vegetalized embryos. We are analyzing now which *T*-genes homologue is responsible for the archenteron formation by *in situ* hybridization and Northern blotting.

FUNCTIONAL ANALYSIS OF PTEN AT GASTRULATION OF *XENOPUS LAEVIS*

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In embryonic development, cells proliferate, and then differenciate. The PTEN, a dual-specific phosphatase that dephosphorylates both proteins and lipids, is an important regulator for both cell cycle and cell migration. It has been reported that PTEN-knockout mice died at approximately E6.5, blastcyst stage, but a role of PTEN gene in embryonic development has not yet been investigated. In this study, we analyzed the function of PTEN gene at a transition from a cell proliferation state to a differentiation state at gastrulation in the frog, *Xenopus laevis*. The mRNA, as well as protein of PTEN were expressed at early embryonic stage. Scince the overexpression of wild-type PTEN inhibits cell migration in fibroblasts, we determined the effect of PTEN overexpression on cell migration during embryonic development. The overexpression of PTEN caused a delay in gastrulation. Furthermore, we determined whether a lipid- or a protein-phosphatase activity is required for the gastrulation by the overexpression of several functional mutants of PTEN. These results indicate that PTEN is involved in cell migration at gastrulation in *Xenopus*.

CHANGES OF THE MITOTIC APPARATUS CAUSED BY THE DISAPPEARANCE OF CHROMOSOMES IN STARFISH EGGS USING THE POLARIZATION MICROSCOPE, LC-POLSCOPE

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In the eggs of the starfish, *Asterina pectinifera*, the mitotic apparatuses during meiosis and cleavage were observed with a polarization microscope equipped with LC-PolScope imaging system (Cambridge Research and Instrumentation, Inc., MA, USA). The retardance of the spindle of the mitotic apparatus was almost the same both at meiosis and cleavage, whereas the asters were quite small at meiosis but large at cleavage in retardance and size. The retardance of the spindle at meiosis decreased and also chromosomes diminished gradually shortly after the injection of bleomycin, an antibiotics which causes DNA strand scission depending on its concentration. When the oocytes were treated with aphidicolin at meiosis, a DNA polymerase inhibitor, DNA synthesis did not occur during cleavage. When the mitotic apparatus in the eggs treated with aphidicolin during cleavage, it looked like that of the control egg at the first cleavage but the spindle was observed until later than that in normal eggs. On the other hand, at the second cleavage, only two asters but not the spindle were observed and then the blastomere cleaved.

GADOLINIUM ION ACTS DIRECTLY ON THE PRIMARY MESENCHYMAL CELLS RESULTING IN THE ASYMMETRIC SPICULE FORMATION OF SEA URCHIN EMBRYOS

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We have shown that an incubation of fertilized sea urchin eggs in filtrated sea water (FSW) containing 0.01-10 μM Gd^{3+} induced the asymmetric formation of spicules in embryos. We examined the possibility that the primary mesenchymal cells (PMC) or matrix on which the crystal growth of $CaCO_3$ took place distributed asymmetrically in the embryos by an indirect fluorescent antibody technique. PMC and matrix were shown to distribute symmetrically on both sides even in the embryos having only one spicule. Next, we examined the possibility that Gd accumulated in embryos interfered the nucleation or elongation of $CaCO_3$ crystal by an X-ray microanalysis. We could not find that Gd accumulated somewhere in the PMC, blastocoel or spicules. Inhibitory effect of Gd^{3+} on spicule elongation is probably not a direct effect on the crystallization of $CaCO_3$. Finally, PMC were isolated from the early blastulae and incubated in FSW containing 2% horse serum and Gd^{3+}. Gd^{3+} inhibited the formation of spicule in a dose-dependent manner. This suggests that Gd^{3+} acts directly on PMC and exerts an inhibitory effect on the spicule formation, but the mechanism of asymmetric inhibition *in vivo* is unknown.

EXPRESSION AND FUNCTION OF SEA URCHIN PUMILIO ORTHOLOG

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Pumilio is a member of Puf family of RNA-binding protein and acts as a translational repressor in embryonic patterning and germline stem cell development. In order to know the role of Pumilio in sea urchin embryogenesis, we clone a cDNA for sea urchin (*Hemicentrotus pulcherriums*) ortholog of Pumilio(HpPum). The *HpPum* cDNA encoded a 1142 amino acid protein containing a highly conserved RNA-binding domain, known as the Puf domain. Northern blot analysis revealed that *HpPum* mRNA existed from unfertilized egg to unhatched blastula, then increased in hatched blastula, reaching a maximum level in mesenchyme blastula, and thereafter declined. Immunostaining with anti-*Xenopus* Pumilio monoclonal antibody showed that HpPum protein increased after hatching and uniformly distributed in embryo. To understand the function of HpPum, we designed the experiment to perturb the embryo by inducing ectopic overexpression of Puf domain of HpPum. The overexpression of Puf domain suppressed the gastrulation. These results suggest the possibility that HpPum-mediated translational regulation is involved in the process of gastrulation.

THE DEVELOPMENTAL PROPERTIES OF THE NUCLEO-CYTOPLASMIC HYBRID BETWEEN LOACH AND GOLDFISH

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In teleost, nucleo-cytoplasmic hybrids have been artificially induced by nuclear transplantation and artificial androgenesis, but little is known about their developmental properties. In this study, androgenetic haploid nucleo-cytoplasmic hybrids were induced by fertilizing UV irradiated loach eggs with goldfish sperm, and developmental capacity of hybrid embryos and embryonic cells was examined. Until the late blastula stage, the hybrid embryos developed normally and expressed the mesodermal marker genes *goosecoid* and *no tail* as in haploid loach embryos. They were arrested before the gastrula stage, although androgenetic haploid loach embryos

survived until hatching. At the gastrula stage in loach embryos, the hybrids showed abnormal appearance and gene expression pattern. When blastomeres of these nucleo-cytoplasmic hybrids were transplanted to diploid loach embryos at the late blastula stage, these cells were mingled with host blastomeres during gastrula stage, and transplanted cells were viable in the loach embryos even in the hatching stage.

ANALYSIS A CELL CYCLE MECHANISM WITH TRANSPARENT BLASTOMERES OF *XENOPUS LAEVIS*

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Early embryonic development in the frog, *Xenopus laevis* is characterized by rapid and synchronous cell cycles. At midblastula transition (MBT), the cell cycle times were elongated by addition of two gap phases (G1 and G2). To analyze a changing mechanim in those cell cycles, transparent blastomeres were produced by removing yolk granules with centrifugation. The transparent blastomeres were stained Hoechst 33342, and measured the fluorescence intensity in nuclear DNA. The exposure of weak UV light with very short periods did not affect the development of transparent blastomeres. The cell cycles lengthened with propotinal continued elongation of S phase, accompanied with appearance of G2 and G1. The transparent blastomere produced in this study in a very useful system for examination of cell cycle events on real time.

HOX GENES OF THE STALKED CRINOID, *METACRINUS ROTUNDUS*

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Stalked crinoids are the most primitive group in the extant echinoderms. Echinoderms are a member of deuterostomes, along with hemichordates and chordates. Thus, stalked crinoids are phylogenetically important for understanding the evolution of the body plan of echinoderms as well as deuterostomes.

Hox genes play an important role in patterning body plans along the anterior-posterior axis, and this patterning mechanism is conserved throughout metazoans.

In order to understand the body plan of stalked crinoids and also the origin of the echinoderms, we isolated *Hox* genes from the stalked crinoid *Metacrinus rotundus*, and examined gene expression patterns in larvae. A PCR-survey revealed that *M. rotundus* has at least eight *Hox* genes: two anterior, four medial, and two posterior *Hox* genes. Among them, the expression of three *Hox* genes were detected in the somatocoels that is formed in the posterior region of early dipleurula-type larvae by whole mount *in situ* hybridization.

A MARINE-SPONGE-DERIVED SUBSTANCE THAT INHIBITS CELL FATE SPECIFICATION DURING SEA URCHIN EMBRYOGENESIS

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A novel spirocyclic sesquiterpene that inhibits cell fate specification during sea urchin embryogenesis has been obtained from a methanolic extract of the marine sponge *Geodia exigua*. The structure of the substance designated exiguamide has been determined to be a derivative of (-)-10-*epi*-axisonitrile-3 the isonitrile functionality of which is replaced by the formlamino group. When fertilized eggs of the sea urchin, *Hemicentrotus pulcherrimus*, were cultured in the presence of 0.4 μM exiguamide, they divided equally to form 16-cell embryos that were comprised of sixteen cells of the same size. In a control experiment, normal embryos formed four macromeres, four micromeres, and eight mesomeres at the same 16-cell stage. After passing through the blastula and then gastrula stages, the treated embryos developed to spicule-deficient plutei. Exiguamide could be a useful tool for elucidating the molecular mechanism of cell fate specification during sea urchin embryogenesis.

STAGES OF EARLY EMBRYONIC DEVELOPMENT IN WILLOW MINNOW, *GNATHOPOGON CAERULESCENS*

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For the successful creation of a germline chimera by transplantation of primordial germ cells (PGCs), it is required the detailed studies of embryogenesis about the donor/recipient individuals. In this study, we introduce a new *Cyprinidae* material, willow minnow *Gnathopogon caerulescens*, for making the germline chimera and for studying PGCs migration. We describe a series of stages for development of the early embryo of the willow minnow, and compare with those in zebrafish embryos. Andmore, we observe the migration route of PGCs by *vas* and *nos-1* as a marker molecule for PGCs.

LOSS-OF-FUNCTION ANALYSIS OF *KRL* GENE OF THE SEA URCHIN *HEMICENTROTUS PULCHERRIMUS*

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We performed a loss-of-function analysis of *HpKrl* gene of the sea urchin *Hemicentrotus pulcherrimus*. Fertilized eggs injected with morpholino antisense oligonucleotides complementary to *HpKrl* mRNA developed almost normally to mesenchyme blastula stage. Micromere descendants ingressed to the blastocoel as the primary mesenchyme cells (PMCs), and formed normal spicules. However, gastrulation was severely retarded. In order to examine micromere functions in *Krl*-knockdown embryos, especially the endoderm inducting activity, we microsurgecally formed chimeras composed of animal cap mesomeres from a normal embryo with the micromere quartet isolated from an injected embryo. In this chimera, micromere descendants differentiated normally to skeletogenic mesenchyme cells via PMCs. However, archenteron was not induced from an animal cap of the chimeric embryo. These observations indicate that *HpKrl* is not required for micromere specification to the skeletogenic mesenchyme cell, but is necessary for the endoderm inducing activity.

DEVELOPMENT OF SCANNING ELECTROCHEMICAL MICROSCOPY TO MEASURE THE RESPIRATION OF MAMMALIAN EMBRYOS

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Scanning electrochemical microscopy (SECM) is a technique in which the tip of a microelectrode is used to scan and monitor the local distribution of electro-active species (oxygen) near the sample surface. We succeeded in non-invasively and quanitatively determining oxygen consumption of individual bovine embryos by SECM. Although SECM can be useful for assessing respiration activity of embryos, the SECM measuring procedure requires quite a bit of skill. Recently, we designed a new SECM measuring procedure which can be easily used by a non-electrochemist. This new SECM measuring system includes a measuring instrument on an inverted optical microscope stage, a potentiostat, and a notebook computer as controller and analyzer. Using this modified procedure, oxygen consumption has been monitored at various developmental stages of single, identical bovine embryos developed from in vitro-matured and fertilized oocytes. Oxygen consumption rates of the single embryos were low from 2-cell to 8-cell stages. An increase in the oxygen consumption rate were found at the morula stage and blastocysts showed an even higher oxygen consumption rate.

IDENTIFICATION AND ANALISIS OF EXPRESSION PATTERN OF HPKRL

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In the sea urchin embryogenesis, nuclear beta-catenin is essential for the formation of vegetal structures. Nuclear entry of beta-catenin is first detected at the 16 cell stage in micromeres, and gradually spreads through the macromere progeny. We previously demonstrated that (1) beta-catenin directly activates *micro1* in the micromere, and (2) *micro1* is necessary and sufficient for micromere specification to the skeletogenic mesenchyme cells. Recently, *SpKrl* was identified as a direct target of beta-catenin from *Strongylocentrotus purpuratus*, which encodes a transcription factor with a Zn-finger DNA-binding motif. In this work we isolated *Hemicentrotus pluchertimus* ortholog, *HpKrl*, and analized the expression patterns in the embryo.

cDNA CLONING OF A VASA-LIKE GENE OF THE GREEN SHORE CRAB, *CARCINUS MAENAS*

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The origin of germ cells and the molecular mechanisms of primordial germcell (PGC) determination in crustaceans is unclear. Vasa is a member of theDEAD (Asp-