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

Shuichi Ueno[1], Yoshio Masui[2], Yasuhiro Iwao[1]
[1]Department of Biological Science, Faculty of Science, Yamaguchi University, Yamaguchi city, Yamaguchi 753-8511, Japan and [2]Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, M5S 3G5, Canada

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?

Kyojy Miyawaki[1,2], Takuya Doihara[1,3], Yuji Miguchi[1,3], Hiroaki Komori[4], Kazuhiro Shigemoto[5], Katsumi Mominoki[6], Masahito Ogasawara[7], Chun-yu Li[1], Jie Chen[1], Shuang-yan Gao[1], Kyoko Saito[1], Takehiro Terashita[1], Tetsuya Shimokawa[1], Shouichiro Saito[1,8], Naoto Kobayashi[1], Seiji Matsuda[1], Masato Nose[4]
[1]Division of Anatomy and Embryology, Department of Integrated Basic Medical Science, Ehime University School of Medicine, [2]Division of Environmental Comparative Pathology, Center of Marine Environmental Studies, Ehime University, [3]Department of Biology and Earth Sciences, Faculty of Science Ehime University, [4]Division of Pathogenomics, Department of Pathology, Ehime University School of Medicine, [5]Division of Ecogenetics, Department of Environmental Health and Social Medicine, [6]Department of Biological Resources, Integrated Center of Science, Ehime University, [7]Division of Pharmacology, Department of Integrated Basic Medical Science, Ehime University School of Medicine and [8]Division of Veterinary Anatomy, Department of Basic Veterinary, Faculty of Applied Biological Science, Gifu University

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*

Rinco Kono, Shuichi Ueno, Yasuhiro Iwao
Department of Biological Science, Faculty of Science, Yamaguchi University, Yamaguchi city 753-8512, Japan

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

Yukihisa Hamaguchi, Miyako S. Hamaguchi, Setsuko K. Satoh
Department of Bioengineering, Graduate School of Biocience and Biotechnology, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8551, Japan

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

Junko Murai[1], Ritsu Kuroda[1], Norihiko Uto[2], Yoshinori Muranaka[3], Hideyo Kuroda[1]
[1]Department of Environmental Biology and Chemistry, Faculty of Science, Toyama University, Toyama, Toyama930-8555, Japan, [2]Laboratory of Biology, Hamamatsu University School of Medicine, Hmamatsu, Shizuoka 431-3192, Japan and [3]Research Equipment Center, Hamamatsu University School of Medicine, Hmamatsu, Shizuoka 431-3192, Japan

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

Hiroka Iida[1], Keiko Mitsunaga-Nakatsubo[1], Ikuya Saito[1], Taishin Shimotori[2], Naoaki Sakamoto[2], Koji Akasaka[2], Takashi Yamamoto[1]
[1]Dept. of Math. and Life Sci., Grad. Sch. of Sci., Hiroshima Univ., Higashi-Hiroshima 739-8526, Japan and [2]Misaki Marine Biological Station, Grad. Sch. of Sci., The Univ. of Tokyo, Kanagawa 238-0225, Japan

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

Takafumi Fujimoto[1], Taiju Saito[1], Suzu Sakao[1], Etsuro Yamaha[2], Katsutoshi Arai[1]
[1]Laboratory of Breeding Science, Graduate School of Fisheies Science, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan and [2]Nanae Fresh Water Laboratory, Field Science Center for Northern Biosphere, Hokkaido University, Nanae, Kameda-gun, Hokkaido 041-1105, Japan

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