M phase. The cytoplasmic [Ca^{2+}] also increased before M phase. In addition, continuous oscillation in small increase of cytoplasmc [Ca^{2+}] was observed during cleavage. The oscillation in cytoplasmic [Ca^{2+}] might be a timer for the blastomere to determine the timing of cell division during cleavage.

FUNCTIONAL ANALYSIS OF PTEN AT GASTRULATION OF *XENOPUS LAEVIS*

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The PTEN phosphatase is an important regulator for both cell cycle and cell migration. It has been reported that PTEN knockout mice died at 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 arround MBT and gastrulation in the frog, *Xenopus laevis*. The first, overexpression of PTEN caused a delay in the progress of gastrulation. The second, we demonstarated immunohistochemically that PTEN began to localize to plasma membranes and nuclear after MBT, and then to nuclei after gastrulation. Since in previous reports, PTEN functions near the plasma membranes, we next analyzed the involvement of changes in PTEN localization at gastrulation. The third, overexpression of membrane localization signal mutated PTEN caused a significant delay in gastrulation and changed phospholylation of Akt. These results indicate that appropriate PTEN activity is necessary for the normal progress in gastrulation, which is probably regulated by differential localization between MBT and gastrulation.

THE REGULATING MECHANISM OF CENTROSOME SEPARATION AFTER MID-BLASTULA TRANSITION (MBT) IN *XENOPUS* EMBRYOS

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The separation of centrosomes occurs at telophase in M phase during the short cell cycles before mid-blastula transition (MBT) when both zygotic gene expression and morphogenesis begin in *Xenopus* embryos, but it occurs during G1/S phase after MBT. To investigate the regulating mechanism of centrosome separation, we observed the behavior of centrin, a major component of pericentriolar materials (PCMs), by using the expression of EGFP-tagged centrin in the translucent blastomeres and by immunochemistry.

The endogenous centrin was detected at centrosomes and a nucleus from interphase to prometaphase, and at spindles from metaphase to telophase before MBT. Larger amount of centrin was accumulated at centrosomes in the embryos after MBT than before MBT. The very small amount of EGFP-centrin was detected before MBT, but larger amount of centrin was localized at centrosome and a nucleus after MBT. These results suggest that there is no significant difference of the localization between endogenous and EGFP-tagged centrin and that the accumulation of centrin at the centrosomes after MBT is involved in the delay of the centrosome separation after MBT.

EARLY DEVELOPMENT OF THE REEF-BUILDING CORAL *ACROPORA*

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Early development of a reef-building coral *Acropora tenuis* (Cnidaria; Anthozoa) was observed. The cleavage was holoblastic despite dense yolk, and unequal spiral cleavages followed to result in irregular forms of the morula and blastula. In the coeloblastula stage yolk granules migrated to the inner side of the embryo in each blastomere, and a certain fraction of granules were once excreted into the blastocoel and retrieved later. Gastrulation occurred not by invagination but in a unique and novel way. In the planula larva an invaginate pocket was formed at the aboral tip, in which numerous spirocysts were contained. It is supposed that deposition of the sticky spirocysts contributes to attachment of the planula onto substrates in initiation of settlement and metamorphosis.

HYBRID MPF CONSISTING OF CDC2 AND CYCLIN B DERIVED FROM DIFFERENT SPECIES IS RESPONSIBLE FOR ABNORMAL EMBRYONIC DEVELOPMENT IN INTER-SPECIFIC HYBRIDS

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Inter-specific hybrids undergo aberrant embryonic development. This phenomenon prohibits the hybrids from growing, thereby playing a critical role in reproductive isolation. However, its molecular basis is still unknown. A hybrid between *Oryzias latipes* and *O. javanicus* is embryonic lethal, since paternal chromosomes are eliminated during embryogenesis. The M-phase-promoting factor (MPF), a complex of cdc2 and cyclin B, is a key regulator of the cell division. Since the hybrid cells contain hybrid MPF consisting of subunits derived from different species, we investigated a possibility that anomalous phosphorylation catalyzed by the hybrid MPF induces the abnormal cell division in the hybrid. Injection of mRNAs encoding *O. latipes* and *O. javanicus* cyclin B into the fertilized eggs of *O. latipes* produced wild-type MPF and hybrid MPF in the injected cells, respectively. *O. latipes* embryos with wild-type MPF underwent normal cell division, whereas those with hybrid MPF exhibited abnormal cell divisions, with the occurrence of chromosome elimination. These results strongly suggest that the hybrid MPF is responsible for the abnormal embryonic development in the hybrid.

ADULT INDIVIDUALS OBTAINED FROM EMBRYOS RECONSTRUCTED BY NUCLEAR TRANSFER OF THE ADULT CAUDAL FIN CELLS IN MEDAKA

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In fish and amphibians, nuclear transplantation of adult somatic cells has made a limited success. In these animals, cell cycle of the egg cytoplasm is remarkably faster than that of donor cells. This cell cycle incompatibility between the recipient egg cytoplasm and donor nuclei results in chromosomal aberrations which cause abnormal development of reconstructed embryos. We have overcome this problem by using nonenucleated eggs as recipients that were diploidized by chromosomal manipulation. We transplanted primary cultured cells from the adult caudal fin of a GFP transgenic medaka strain to diploidized recipient eggs. About 1% of reconstructed embryos developed to adult individuals, which were fertile and diploid, correctly expressed GFP donor marker as in the donor strain, and transmitted the marker to F1 and F2 generations in a Mendelian fashion. These results in medaka open the way to development of cloning and gene targeting technologies in fish and shed light on studies of reprogramming of differentiated cells and development of reconstructed embryos by nuclear transfer not only in fish, but also in amphibians and mammals.

***KRUPPEL-LIKE* PARTICIPATES IN SPECIFIATION OF THE SEA URCHIN MICROMERE**

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We have reported that *micro1* gene plays central roles in micromere specification in the sea urchin embryo. However, *micro1* is not sufficient for induction of the secondary mesenchyme cell (SMC). Nuclear β-catenin directly activates both *micro1* and *Kruppel-like* (*Krl*) in micromeres. Here, we show that *Krl* participates in production of the inductive signal(s). Krl-knockdown micromeres were deficient in SMC-inducing activity, indicating that *micro1* and *Krl* are complementary in induction of endomesoderm structures. Conversely, ectopic expression of *Krl* in animal blastomeres not only resulted in formation of endomesodermal tissues but also endowed them with the ability to function as an organizing center.

FUNCTIONS OF THE *KRUPPEL-LIKE* GENE IN THE SEA URCHIN MACROMERE

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Micro1 and *Kruppel-like* (*Krl*) have been identified as the targets of nuclearβ-catenin. Although *micro1* expression is restricted to micromeres, *Krl* is activated gradually in vegetal halves according to nuclear localization of β-catenin. *Krl*-knockdown embryos which had been injected with morpholino antisense oligonucleotides complementary to *Krl* mRNA developed almost normally to the mesenchyme blastula stage. Micromere descendants ingressed to the blastocoel as the primary mesenchyme cells (PMC), and formed normal spicules. However, gastrulation was severely retarded and the secondary mesenchyme cells (SMC) formation was blocked. In order to illuminate *Krl* functions in the macromere lineage, we microsurgecally formed chimeric embryos and analyzed the phenotypes, which were composed of normal blastmeres and experimental ones. The analyses indicate that *Krl* is required in macromeres for cell-autonomous formation of endomesodermal structures, but not for reception of micromere-derived signals. We also show that there exist two distinct pathways to SMC formation, *Krl*-dependent and *Krl*-independent ones.

CONTRIBUTION OF HYDRO PORECANAL AND LEFT ANTERIOR COELOM ON EXPANSION OF LARVAL BODY

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In normal development of starfish, the posterior end of the left anterior coelom (lac) forms hydro porecanal (hpc), which opens to exterior on the dorsal side of bipinnariae. Removal of the entire lac including hpc suppresses expansion of larval body after hpc formation stage. In order to examine the contribution of lac and hpc on expansion of larval

body, partial deletion of lac area was carried out. Removal of hpc alone resulted in suppression of larval body expansion, while removal of lac leaving intact hpc resulted in incomplete suppression. These results suggest that both hpc and lac contribute to expansion of larval body, and that lac requires hpc for its involvement in larval body expansion.

FUNCTION OF MEIOTIC ASTRAL MICROTUBULES IN THE STARFISH OOCYTE

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Germinal vesicle breakdown and the release of the first and second polar body occur after 1-methyladenine treatment in the starfish maturing oocyte. During the process of meiosis, the meiotic apparatus locates to the animal pole. The aster, the structure containing radial microtubules, exists in both poles of the meiotic apparatus. It was reported that the strong fluorescence is observed in the peripheral half spindle by immunofluorescence (Satoh, et al, Dev. Growth & Differ.,36 (6),557-565 (1994)). Thereby, it is considered that the aster plays very important roles in meiosis. However, the structure of the aster is not clear. In this study, we researched the details of the astral structure and the change of the cortical aster by immunofluorescence. We discussed the function of the astral microtubules during meiosis.

THE DIRECTIONS OF POLARIZED CYTOPLASMIC MOVEMENT AND THE FIRST CLEAVAGE PLANE ARE SHIFTED BY CENTRIFUGATION IN STARFISH EGGS

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The polarized cytoplasmic movement occurs along the animal-vegetal axis prior to the first polar body formation in the oocytes of the starfish, *Asterina pectinifera*. Subsequently, the first cleavage plane forms along the animal-vegetal axis. We reported at the last meeting that the direction of the polarized cytoplasmic movement was shifted toward centripetal direction by centrifugation whose axis was perpendicular to the animal-vegetal axis during early meiosis I. In this report, the first cleavage plane in the centrifuged eggs was shifted toward the centripetal direction. Its direction also consisted with the direction of the polarized cytoplasmic movement. This result suggests that both directions of the polarized cytoplasmic movement and the first cleavage plane were determined by the factors which were shifted by centrifugation.

DESCRIPTION OF EARLY CLEAVAGE PATTERN OF THE LARVACEAN, *OIKOPLEURA DIOICA*

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Oikopleura dioica is a urochordate, and closely related to ascidians. It has many advantages for biological research, such as a short life cycle (4days at 18°C) and small cell number,In order to use this organism as an experimental animal for developmental biology, it would be important to understand early cleavage pattern of the embryos. In this study, we carried out detailed observation of early cleavage pattern of *O.dioica* embryos, and described each blastomeres up to the 32-cell stages, We also found that the most posterior blastomeres cleave quite unequally from the 8-cell to 32-cell stages as is known in ascidian embryos.

LOCALIZATION OF β-CATENIN PROTEIN IN EARLY EMBRYOGENESIS OF ASCIDIAN *HALOCYNTHIA RORETZI*

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Localization of proteins plays important roles in axis formation and cell fate specification during embryogenesis. In sea urchins and the ascidian *Ciona*, β-catenin protein is transported into nuclei in only vegetal hemisphere, and functions to establish the animal-vegetal axis formation. In this study, we observed localization of β-catenin protein in early embryogenesis of the ascidian *H. roretzi* with the specific anti body against *H. roretzi* β-catenin. The protein was localized in the nuclei in endoderm cells of the vegetal hemisphere. We also observed localization of Dshevelled protein which involves β-catenin signal pathway with the specific anti body.

DEVELOPMENT OF THE LEFT-RIGHT SEPARATED BLASTOMERES OF ASCIDIAN EMBRYO

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Ascidian larvae have left-right asymmetry. For example, photoreceptor cells are localized on only right-lateral side. Ascidian egg is well known mosaic character. To examine whether or not the different development between left and right blastomeres is determined at the 2-cell stage, we separated the blastomeres at 2-cell stage and observed the following development. Until gastrula stage, the embryos exhibited the shapes like the hemisphere of the normal embryo. However, the embryo was deformed after neurula stage. Some deformed embryos developed bent tails and produced pigments like otolith and ocellus. The embryos 24 hours after fertilization, the embryos were fixed and immunostained with anti-Ci-Arr antibody to examine the differentiation into photoreceptor cells. In most case, one of the separated blastomere generated Arr-positive cells and the other did not. This result shows that the fates of left and right blastomeres are already determined at 2-cell stage.

DIFFERENTIATION OF PRIMORDIAL GERM CELLS FROM EGG IRRADIATED WITH ULTRAVIOLET RAY BEFORE FERTILIZATION IN THE LOACH

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In amphibians and *Drosophila*, UV irradiation to the eggs prevents a subsequent differentiation of primordial germ cells (PGCs). In teleost, effect of UV irradiation on formation of PGCs during embryogenesis still remains unclear although functional gametes have been formed in androgenic progeny which develop from UV irradiated eggs fertilized with normal sperm. In this study, we examined formation of PGCs in the haploid loach embryos induced androgenetically. The number of PGCs in androgenic haploid embryos was statistically reduced, when compared with gynogenic haploid and normal diploid ones at the late-blastula stage. PGCs proliferated at the germ ring stage, and then located in putative gonadal anlagen at 5 days after fertilization in androgenic haploid embryos as observed in control ones. When PGCs of androgenic haploid were transplanted into blastoderm of diploid embryos at the late blastula stage, donor PGCs were observed in the gonadal anlagen of host larvae. Consequently, UV irradiation reduces the PGCs number at the blastula stage, but not affects on the functions of PGCs, such as proliferation and migration.

THE ROLE OF DNA METHYLATION IN THE EARLY DEVELOPMENT OF *CIONA INTESTINALIS*

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DNA methylation is one of the epigenetic factors which affect the level of chromatin folding and thus contribute to gene silencing. It has been clearly shown that DNA methylation plays an important role in cell differentiation. The genome and cDNA projects in *Ciona intestinalis* have released draft genome sequence, a set of ESTs and cDNA sequences and genemodels, which enable us to adopt various approach to *Ciona* development. However, the role of DNA methylation in the early development of *Ciona* is scarcely studied. In this study, we blocked DNA methylation by treatment of embryos with 5-aza-deoxycytidine. DNA-methylation-blocked embryos developed normally until the start of gastrulation, however the development began to delay, ceased at the neurula stage. They gradually deformed because of apoptosis and finally died. We collected these embryos arrested at the neurula stage, extracted total RNA from them and used it for the microarray analysis.

IDENTIFICATION AND ANALYSIS OF EXPRESSION PATTERN AND FUNCTION OF HpaPKC

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aPKC and PAR proteins are involved in asymmetric cell division and establishment of cell polarity. Here we isolated HpaPKC from the sea urchin *Hemicentrotus pulcherrimus*, and examined its expression and function. Maternal HpaPKC transcrips were uniformly distributed to the early blastula stage, while zygotic ones were restricted to micromere descendants. Embryos in which aPKC functions had been suppressed with the morpholino antisense oligonucleotide or the dominant negative form of mutation developed normally to the mesenchyme blastula stage. However, subsequent gastrulation and skeletogenesis were defective in the embryo. When combined with animal halves, aPKC-knockdown micromere descendants ingress as the primary mesenchyme cells, but did not induce ectopic gut. These observations suggest that aPKC is involved in PMC specification and production of inductive signals.