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-