

VYC-removed embryos operated at blastula stage, although VYC deletion at the 1-cell stage causes the anterodorsal deficiencies. These results suggest that the yolk cell is essential for the trunk-tail morphogenesis after gastrulation in zebrafish.

THE ROLE OF TAILBUD IN THE TAIL FORMATION IN ZEBRAFISH

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The cell proliferation in tail bud seems to contribute to the development of the tail in zebrafish. Moreover, previous studies suggest that the ventral marginal cells of the gastrula induce the tail formation. This region contains cells that are normally fated to form part of the tailbud, although inductive activity of ventral marginal cells significantly decreases until the end of gastrulation. These studies raise the question of whether tailbud completely lose the ability to induce the tail. In order to test the inductive activity of marginal cells after the gastrulation, we conducted a transplantation experiment. The tailbud isolated from donor embryo at 18-31 somites stage was transplanted into the animal pole region of the host embryo at blastula stage. The most obvious effect of grafts was the formation of ectopic secondary tail. The secondary tail contained the somite-like structures or myotomes. In the extreme case, the secondary tail was comparable in size to 50% of the total length of host embryo. Our results suggest that the tailbud region keeps the ability to form a secondary tail from gastrulation stage and throughout somitogenesis.

PRESSURE RESPONSE OF MEDAKA EMBRYOS I-APOPTOSIS WITH TIME-LAG-

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Embryogenesis of medaka was studied by tracing the pressure response of medaka embryo (inbred strain Hd-rR). Embryos were pressurized at a pressure of 20-140 MPa for 5 min. In various stages, development was delayed after depressurization. Embryos pressurized before blastula stage died with diffused apoptotic cells though they could be the stage of blastula. Embryos pressurized before neural plate formation could make a neural plate, and induced apoptotic cells in a limited area of the midbrain about 10 hours after decompression. Some embryos survived after detection of apoptosis could hatch with various anomalies including an ocular defect.

PRESSURE RESPONSE OF MEDAKA EMBRYOS II -ANALYSIS OF PROTEIN EXPRESSIONS BY 2-DIMENSIONAL GEL ELECTROPHORESIS-

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Embryogenesis of medaka was studied by tracing the pressure response of medaka embryo (inbred strain HNI). Embryos were pressurized in early stages (stage 8, 12 and 16) at a pressure of 100 MPa for 5 min. After depressurization, time-series of protein expression were measured by 2-dimensional gel electrophoresis. Expression of various proteins including β -actin, α -tubulin, β -tubulin, vitellogenin and protein kinase C revealed to respond to the pressure operation. Stage dependence of expression of each identified protein was examined in comparison to that of the non-pressurized control.

ANALYSIS OF THE FUNCTION OF Hox GENES IN *CIONA INTESTINALIS*

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Hox genes have been noted to play a central role in anterior-posterior patterning of the animal body. Draft genome analysis of the ascidian *Ciona intestinalis* has identified nine Hox genes and our recent study has shown that they are localized on two chromosomes in the genome and their colinearity is residual. Their functions in the development, however, are still unknown. We have started investigation of the function of Hox genes in *Ciona intestinalis*. For this purpose, we prepared MOs (Antisense Morpholino Oligonucleotides) against eight *Ciona* Hox genes, of which expression has been detected by whole-mount *in situ* hybridization during embryogenesis and/or metamorphosis, and injected them into *Ciona* eggs. Here we report some of the results obtained through this approach. Knockdown of *Ci-Hox12* resulted in the abnormal morphology and change in the expression level of some developmental marker genes at the tail tip of the embryo at the middle to late tail bud stages. Translational inhibition of *Ci-Hox10* resulted in the lack of whole intestine of the juvenile. These results suggest for the first time that some of Hox genes play essential roles in the development of ascidians.

CONSERVATION OF TRANSCRIPTIONAL REGULATION OF Lhx3 GENE IN TWO ASCIDIAN SPECIES

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Embryonic development looks very similar between two phylogenetically remote ascidian species, *Halocynthia roretzi* and *Ciona intestinalis*. We are interested in examining transcriptional regulatory mechanisms of developmental genes between two species. In the development of ascidians, *Lhx3*, a LIM homeodomain transcription factor gene is expressed in the cells of endodermal lineage and notochordal lineage before and during gastrulation and some cells in the central nervous system at the tailbud stage. Previously, we reported a cDNA sequence for *Lhx3* of *H. roretzi*. In the present study, we carried out 5' RACE with RNA from cleavage stage embryos of *H. roretzi* and found that a sequence different from that we previously reported was present among 5' RACE products. The new sequence was located in the intron between exons encoding the previously identified 5' most sequence and the first LIM domain. We also carried out 5' RACE using cleavage stage RNA of *C. intestinalis*, which revealed that a similar situation is present to that observed in *H. roretzi*. These results suggest that transcriptional regulation of *Lhx3* during development may be conserved between the ascidians.

MOLECULAR MECHANISMS REGULATING MORPHOLOGICAL DIVERSITY IN TOOTH DEVELOPMENT

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Many genes regulating the tooth development have been reported, and studies only based on rodents such as mice have been published so far. Murine dentition represents specialized morphology, only incisors and molars, although the tooth morphogenesis display significant morphological and developmental differences among species. So, we need other experimental animals than mice for analysis of the tooth development. *Suncus murinus* is a species of the order Insectivora, which has been considered as primitive and one of the earliest eutheria phylogenetically. The *Suncus* has all tooth types, i.e. incisors, canine, premolars and molars. However, in the *Suncus* cDNA cloning and the expression pattern of various developmental regulatory genes have been poorly reported. In this study, we identified several molecular markers, and investigated the expression pattern of various developmental regulatory genes in the *Suncus*.

ROLE OF CXCR4/SDF-1 SIGNALING IN *XENOPUS* GASTRULATION

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Activin is well known as mesoderm and endoderm inducer. The microarray screening using activin-treated and -untreated presumptive ectoderms led us to isolate several novel activin response genes expressing until pre-gastrula stage. *Xenopus* CXC type chemokine receptor 4 (CXCR4) is one of them. CXCR4 has been identified a receptor of Stromal cell derived factor (SDF)-1. Although the various biological functions of CXCR4/SDF-1 signaling were reported, a major function consists of regulator of cell trafficking as CXCR4 positive cells attracted by SDF-1. Therefore, we tried to investigate the role of CXCR4/SDF-1 signaling in *Xenopus* gastrulation. *Xenopus* CXCR4 expression was detected in involuting marginal zone, especially in endomesoderm region, at early gastrula stage, while *Xenopus* SDF-1 α complementally in inner surface of blastocoel roof. Interestingly, region-specific defects of gastrulation were detected in the embryos lacking xCXCR4 and/or xSDF-1 α without effect of convergent extension and mesoderm induction. Taken together with these results, we conclude that CXCR4/SDF-1 signaling regulates guidance of involuting marginal zone in gastrulation.

COMPARISON OF INDUCTION DURING DEVELOPMENT BETWEEN *XENOPUS TROPICALIS* AND *XENOPUS LAEVIS*

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Several *in vitro* systems exist for the induction of animal caps using growth factors such as activin. Here, we compared the competence of activin-treated animal cap cells dissected from the late blastulae of *Xenopus tropicalis* and *Xenopus laevis*. The resultant tissue explants from both species differentiated into mesodermal and endodermal tissues in a dose-dependent manner. In addition, RT-PCR analysis revealed that organizer and mesoderm markers were expressed in a similar temporal and dose-dependent manner in tissues from both organisms. These results indicate that animal cap cells from *Xenopus tropicalis* have the same competence in response to activin as those from *Xenopus laevis*.