MOLECULAR MECHANISM OF LOSS OF THE COMPETENCE IN ASCIDIAN NOTOCHORD INDUCTION

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Notochord cells are induced by FGF at the 32-cell stage in embryos of the ascidian, *Halocynthia roretzi*. When the presumptive notochord cells were isolated at the early 32-cell stage, and treatment with bFGF protein was initiated after various time intervals, the presumptive notochord cells lost the competence to be induced if the treatment was started 120 minutes after the initiation of the 32-cell stage. To understand the mechanism of the loss of the competence, we examined how far FGF signal is transmitted in the signaling cascade by detecting *brachyury* mRNA and activated MAPK. The result shows that MAPK is activated, but that no transcription of the *brachyury* gene takes place in the cells after the loss of the competence.

THE RELATION OF PIGMENTATION AND MIGRATION ACTIVITIES DURING DEVELOPMENT IN CHICKEN MELANOCYTES: ROLE OF ENDOTHELINS AND THOSE RECEPTORS

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To analyze pigmentation mechanism during development of several chicken lines such as Silky, White Leghorn, Nagoya Cochin and albino (c^a/c^a), *etc.*, we have examined the correlation between the expression of pigmentation related genes (*ET3, EDNRB2, c-kit, SCF, integrin-β1, -α4, -α5*, and *Wnt3a*) and migration activities of melanocytes. Our previous studies showed that endothelins (ETs) effected on adhesion on substratum, migration, proliferation and differentiation of melanoblasts/cytes *in vitro* and that fibroblast-like cells and melanocytes expressed *ET3* and the receptor (*EDNRB2*), respectively. In this study, quantitative PCR analysis revealed significant differences on *ET3* and *EDNRB2* between these lines. Especially, *ET3* in Silky was expressed close to twice compared to the other lines at st.18 and the high level continued to hatch. Also Silky expressed high dose of *EDNRB2* at later stages and finally quite high dose of it in muscle and integument before hatch. The antagonists against these EDNRs inhibited melanocytes migration from neural crests in organ cultures. The expression of ET3 before migration was the first and a key process for the melanization in chickens.

ANALYSIS OF FETAL HEPATOBLAST-NONPARENCHYMAL CELL INTERACTIONS BY USING PRIMARY CELL CULTURE SYSTEM

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Cell-cell interactions between parenchymal cells and nonparenchymal cells are important for nomal liver development. However, precise mechanisms of the cell-cell interactions are unknown. Hepatic parenchymal cells in fetal stages are hepatoblasts that differentiate into hepatocytes or cholangiocytes. Nonparenchymal cells contain stellate cells, sinusoidal endothelial cells and Kupffer cells. To analyze the action of nonparenchymal cells in liver development, we examined cell-cell interactions of fetal mouse liver cells in primary culture system. In culture, hepatoblasts formed a monolayer sheet, under which stellate cells were located and showed high proliferating activity. Some sinusoidal endothelial cells were observed on a hepatoblast sheet. Sinusoidal endothelial cells locate closely to stellate cells and hepatoblasts in vivo, implying that they may act on maturation or proliferation of hepatoblasts and stellate cells. To confirm this idea, we compared fetal liver cell cultures with or without sinusoidal endothelial cells.

EXPRESSION OF *Hox1* GENE IN THE POND SNAIL, *LYMNAEA STAGNALIS*

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It is well known that the transcription factors, *Hox* genes, play a critical role in patterning animal body plans along anteroposterior axis. In the gastropod mollusc *Haliotis asinina*, *Hox1* and *Hox4* have been reported to be expressed around the shell gland, suggesting that those genes could be related to the shell formation (Hinman et al., 2003) rather than to the body plan along the anteroposterior axis. Our PCR survey, however, indicated the possible loss of *Hox4* gene in the genome of the gastropod *Lymnaea stagnalis*. This result suggests that the molecular mechanism for the shell formation could be different between two species examined. On the other hand, the fragment of *L. stagnalis Hox1* gene was successfully isolated using the method of 3'-rapid amplification of cDNA ends (3'RACE). We report expression patterns of *Hox1* gene during the development of *L. stagnalis* studied by *in situ* hybridization, and discuss the relationships between shell formation and *Hox* genes in molluscan evolution.

DOWNREGULATION OF NOTCH, MEDIATED BY NUMB AND -ADAPTIN, IS REQUIRED FOR THE DIFFERENTIATION OF POSTEMBRYONIC NEURAL STEM CELLS IN *DROSOPHILA*

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In Drosophila, the postembryonically reactivated neural stem cells (pNSCs) in the neuroepithelium (NE) of the outer optic anlage of the larval brain are distinctive in that they proliferate by symmetric division to be converted into the spherical postembryonic neuroblasts (pNbs) and then divide asymmetrically to give rise to neural precursors that in turn differentiate into neurons and/or glia during the late larval stage.We have investigated how Notch signaling is involved in proliferation and/or differentiation of Drosophila pNSCs along with modulators that regulate the activity of Notch signalling by genetic clonal analyses. We found that Notch is highly expressed in pNSCs and pNbs and is required for both pNSCs and pNbs to proliferate, whereas Numb and alpha-Adaptin, which recruits Notch into AP2 endocytic complex by associating with Numb, are highly expressed both in the pNbs and neural precursors and is necessary for the downregulation of Notch in the pNbs and neural presursors. These results together with biochemical analyses strongly suggest that endocytic downregulation of Notch plays a crucial role for pNSCs to switch from proliferation to differentiation.

SCREENING FOR TRANSCRIPTION FACTORS REGULATING THE ZEBRAFISH PINEAL PHOTORECEPTOR CELL DIFFERENTIATION

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Morphological and biochemical similarities between the pineal and retinal photoreceptor cells indicate their evolutionary kinship. Molecular comparison of their developmental and physiological processes provides important clues to a mechanism underlying functional diversification of vertebrate photosensory systems. We established a procedure for isolating a pure population of the zebrafish pineal photoreceptor cells and that of the retinal rod cells by using fluorescence-activated cell sorting (FACS). We compared gene expression profiles of the sorted cells by ordered differential display, and identified a number of genes expressed selectively in the pineal photoreceptor cells. To reveal a molecular mechanism for pineal-specific gene expression, we focused on two pineal genes encoding functionally uncharacterized transcription factors. Knock-down of each gene using morpholino antisense oligonucleotides led to a significant reduction in expression of *exo-rhodopsin* gene, which serves as a specific marker for the pineal photoreceptor cells. These results demonstrated fundamental roles of the transcription factors in the pineal photoreceptor cell differentiation.

REGENERATION ABILITY OF THE MECHANOSENOSENSORY ORGANS IN THE ARROW WORM, *PARASPADELLA GOTOI* (CHAETOGNATHA)

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Chaetognaths are provided with the mechanosensory organ called a "ciliary fence receptor" (CFR), which consists of fans of elongate cilia. The CFRs are regularly distributed on the body and six CRFs are located at the distal end of the caudal fin of *Parasapdella gotoi*. We paid attention to the six CRFs and examined the re-gen-eration of the fin and the CRFs after cutting the fin. Since the CFRs and the sensory nerves show im-munoreac-tivity to the anti-aspartate antibody, the regen-eration process of the CFRs can be examined by immunocytochem-ical staining. The CRFs were recognized at about 7 days postlesion. Although the number of newly de-veloped CFRs increased, the arrange-ment of the CFRs in the re-generated fin was not differ-ent from that in an intact fin. In the present study we examined the regeneration ability to the successive amputations by using juveniles. The regeneration period of the CRFs in the juveniles was less required than that in the adults. The fins could regenerate even after the third amputation. However, the arrangement of the CRFs was disordered, indicating that the regeneration ability of the CRFs becomes lower after repeated regeneration.

BEHAVIOR OF GERM CELLS DURING SEXUALIZATION IN *ENCHYTRAEUS JAPONENSIS*

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Enchytraeus japonensis can regenerate even from two segments fragments. Although it usually reproduces asexually by fragmentation and regeneration, it can also propagate sexually under a low population density. It is hypothesized that germ cells also regenerate in this species, because any segment of the body can regenerate into a sexualized worm. It is interesting to know the origin of germ cells in regeneration.

Tadokoro *et al.* supposed that the germ cells are originated from the *piwi*-positive cells distributed widely in the body. Some of them migrate to the prospective gonadal regions along with the formation of a head.