pigment granules, pigment cells could be observed under an ordinary microscope as early as the mesenchyme blastula stage. Before the onset of gastrulation, pigment cells were arranged in a hemi-circle (dorsal half) at the central region of the vegetal plate. Bending of the vegetal plate first occurred just at the position occupied by pigment cells. Rhodamine-phalloidin staining showed that actin filaments were abundant at the apical corticies of pigment cells. It was found that the initiation of primary invagination was drastically delayed in the $NiCl_2$ - treated embryos, in which pigment cells were greatly reduced in number. Once invagination started, gastrulation proceeded in a typical manner even in the $NiCl_2$ - treated embryos, i.e., secondary invagination follows with a short lag phase after primary invagination. These show that pigment cells are the bottle cells that trigger the onset of gastrulation, at least in *Echinometra mathaei*.

INVOLVEMENT OF HEDGEHOG SIGNAL TRANSDUCTION PATHWAY IN APICAL GANGLION DIFFERENTIATION IN SEA URCHIN LARVAE

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Hedgehog (Hh) signal transduction pathway plays an important role for valid organogenesis including neurogenesis. To elucidate whether Hh signal transduction pathway participates in the apical ganglion differentiation in sea urchin larvae, we have cloned a hedgehog homologous gene from plutei of the sea urchin Hemicentrotus pulcherimus (HpHh). The amino acid sequence of HpHh had high homology with that of Lytechinus variegates (LvHh). Cyclopamine, an inhibitor of Hh signal transduction to Smo through Patched was administrated from swimming blastula stage to the late gastrula stage. Cyclopamine was effective only at blastula stage when Hh protein was expressed. The treatment suppressed serotonin cell differentiation. We also partially cloned Patched (HpPtc) from the same developmental stage as that HpHh has been cloned. These present observation strongly suggested that HpHh plays an important role for differentiation of neural ganglion in sea urchin larvae.

EXPRESSION ANALYSIS OF HpNeuroD, A NEURO-DIFFERENTIATION-RELATED bHLH TRANSCRIPTION FACTOR HOMOLOGUE, IN PLUTEUS LARVA OF THE SEA URCHIN.

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We have studied molecular mechanism of neuroplexus formation at the oral lobe of pluteus larvae of the sea urchin, *Hemicentrotus pulcherrimus*, and cloned sea urchin homologue of NeuroD, a bHLH transcription factor. The factor has known to be involved in neural differentiation in vertebrates. We have named the sea urchin homologue HpNeuroD, which comprised 421 amino acid residues. Over all amino acid sequence of HpNeuroD was approximately 30% homologous to the known vertebrate's protein, and approximately 80% homologous in conserved region. Phylogenic tree constructed by neighbor-joining method suggested that HpNeuroD belonged to NeuroD1 family. We also have raised anti-HpNeuroD antiserum using a peptide whose amino acid sequence was deduced from a part of the DNA sequence. Immunoblotting showed the antiserum binding band at about 46kDa region that was quite similar to presumed molecular weight. Immunohistochemistry and *in situ* hybridization indicated that HpNeuroD involved in serotonergic neuroplexus differentiation. These results suggested that HpNeuroD is a useful neuronal marker molecule to study neuroplexus formation in sea urchin plutei.

ROLE OF SEROTONERGIC NERVOUS SYSTEM IN SEA URCHIN LARVAL BEHAVIOR AND EXPRESSION OF SEROTONIN RECEPTOR GENE DURING EMBRYOGENESIS

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Sea urchin larva has a serotonergic apical ganglion (SAG). The neurotransmitter has been implicated to regulate swimming behavior based on our present observations of p-chlorophenylalanine (CPA), a serotonin synthetase inhibitor, -treated larvae. To know serotonin administration sites, serotonin receptor gene has been searched, and cloned from the sea urchin, Hemicentrotus pulcherrimus (HpSR). The amino acids sequence of HpSR deduced from the cDNA sequence was phylogenetically located the protein at quite close position to that of rat. Whole-mount in situ hybridization combined with TSA-system (T-WISH) located the message transcription sites at a part of cells in the ciliated band and blastocoelic cells that form serotonin receptor cell network. We also have observed that CPA does not inhibit ciliary movement despite the occurrence of severe perturbation of larval swimming behavior, implicating the presence of serotonin signal mediating/integrating mechanism, such as a serotonin receptor cell network we have reported before, between ciliary beating and swimming behavior.

ESSENTIAL ROLE OF SEROTONIN IN THE FORMATION OF SEROTONIN RECEPTOR CELL NETWORK IN SEA URCHIN PLUTEI.

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We have isolated and sequenced a serotonin receptor gene from the sea urchin, *Hemicentrotus pulcherrimus*, and raised anti-serum against the peptide whose amino acid sequence was deduced from the DNA sequence. p-Chlorophenylalanine (CPA), an inhibitor of tryptophan 5-hydroxylase activity, perturbs swimming behavior of larvae by retaining ciliary movement. To elucidate above apparent discrepancy, we have examined the formation of serotonin receptor cell network in CPA-treated larvae. Relative amount of serotonin receptor protein in these larvae was not significantly affected by CPA. However, immunohistochemistry showed that the formation of network was considerably perturbed by frequent disconnections among serotonin receptor cells. External serotonin application to CPA-treated larvae significantly restored the inter-cellular connections and swimming activity, indicating the formation of serotonin receptor cell network requires serotonin, and implicating serotonin regulates ciliary movement that consequences larval swimming by not directly stimulating ciliary cells, but through serotonin receptor cell network.

PIGMENT CELL CONFORMATION IN ZEBRAFISH STRIPE

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Zebrafish have a characteristic horizontal-stripe pigment pattern made by a specific distribution of three types of pigment cells: melanophores, xanthophores and iridophores. This pattern is a valuable model to investigate how the spatial patterns form during animal development. Recent findings suggest that interactions among the pigment cells play important roles to develop the stripe pattern in zebrafish. However, it is not yet studied minutely.

Therefore, we performed transmission electron microscopic study (TEM) to show the distribution, conformation and how the cells contact with each other in the hypodermis. We found that the pigment cells form complex but ordered layered structures in both stripe and interstripe regions. The order of the layered structures is kept strictly all through the hypodermal regions.

On the basis of the data obtained from the TEM study, we are trying to characterize the behaviors of pigment cells by laser ablation. Also, TEM observation in pigment pattern mutants as well as striped fins of wild type fish is in progress.

Taking with all these results, the mechanisms of zebrafish pigment pattern formation will be discussed.

THE ROLES OF *SHH* IN THE PAIRED APPENDAGES OF THE CARTILAGINOUS FISHES

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In higher vertebrates, restricted expression of *shh* in the posterior margin of the paired appendage primordia is essential for the antero-posterior patterning in the limb or fin buds. On the other hand, in a dogfish, *Scyliorhinus canicula*, no *shh* expression could be detectable in the pectoral and the pelvic fin buds by in situ hybridization and the RT-PCR (Tanaka et al., 2002), suggesting that *shh* has no role in the paired appendages in the cartilaginous fishes. In this study, however, we show that *shh* is expressed in the posterior region of fin buds of two cartilaginous fishes, *Raja kenojei* (a skate) and *Scyliorhinus torazame* (a dogfish) at least in the later stages of development. Different results in terms of *shh* expression between two species of dogfishes are probably caused by the difference of the developmental stages observed. Since *shh* expression of higher vertebrates begins at early stages of limb initiation, later expressions of *shh* may reflect the limited roles of *shh* in the paired appendages of the cartilaginous fishes.

LEFT-RIGHT ASYMMETRICAL CROSSING OF RETINAL GANGLION CELL AXONS IN FLOUNDERS

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It has been reported that flounders have species-specific left-right (LR) asymmetry of retinal ganglion cell (RCG) axons decussating at the optic chiasm. However, the detailed mechanisms for establishing the LR asymmetry remain uncertain. We examined the optic chiasm in some species of teleost fishes, including medaka, zebrafish, and Japanese flounders. All specimens of a Japanese flounder, *Paralichthys olivaceus*, showed the LR asymmetry as described previously; a bundle of RCG