ANALYSIS OF THE VERTEBRAL LIGAMENT FORMATION IN LIVING TRANSGENIC MEDAKA

Keiji Inohaya, Akira Kudo
Department of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8501, Japan

In higher vertebrate, the sclerotome yields materials for the vertebral column and ribs. However, it is not clear whether the sclerotome of the teleost participates in the formation of the axial skeletal structures. To clalify the sclerotome development in the teleost, we have succeeded in generating a transgenic medaka that enables us to trace the behavior of the sclerotomal cells by green fluorescent protein (GFP) in living specimens. The transgenic lines were established by microinjection of a construct containing a putative promoter region of the medaka *twist* gene. In living transgenic medaka, we could observe the head skeletal development as well as the migration of the sclerotomal cells to the neural tube and the notochord. Interestingly, the GFP-positive cells were to be distributed around the putative intervertebral regions during the embryogenesis. The GFP-signals continuously appeared around the intervertebral ligaments of the adult fish. These results showed that the sclerotome-derived cells participate in the formation and the maintenance of the vertebral ligament.

LOCALIZATION AND MIGRATION OF FOLLICULAR MELANOCYTE PRECURSORS IN MOUSE VIBRISSAE DURING HAIR CYCLE

Machiko Iida, Setsunosuke Ihara, Takashi Matsuzaki
Graduate School of Life and Environmental Science, Shimane University, Matsue, Shimane 690-8504, Japan

Follicular melanocyte stem cells will reside in the follicle and supply precursors of differentiated melanocytes into the hair bulb throughout whole life, since follicular melanocytes in hair bulbs die and regenerate as the hair cycle proceeds. Thus we examined localization of melanocyte differentiation antigens, TRP1 and TRP2/Dct, at different stages of mouse vibrissa follicles using immnohistochemical methods in order to describe the process of melanocyte differentiation during hair cycle. Both of these antigens were detected in lower ORS and hair bulb. Although the localization of TRP2(+) cells remained almost unchanged during hair cycle, expression pattern of TRP1 varied among the follicles of different stages. TRP1(+) cells were frequently detected in lower ORS at early anagen, sporadically at mid anagen, and hardly at late anagen. After mid anagen, TRP1(+) cells localized around dermal papillae, which continued until late anagen and disappeared at telogen. These results suggest that melanocyte stem cells would reside in middle portion of the hair follicle and their descendants migrate downward to supply mature melanocytes in the hair bulb every hair cycle.

THE APPEARANCE OF TWO PEAKS OF APOPTOSIS SIGNALS IN SEA URCHINS DURING BLASTULA AND GASTURULA STAGES

Hazime Mizoguchi[1], Shinobu Kawai[2], Akiya Hino[2]
[1]Laboratory of Life Science, Faculty of Social Welfare, Rissho University, Saitama, Kumagaya 360-0194 and [2]Department of Biological Sciences, School of Science, Kanagawa University, Hiratsuka 259-1293

Using the Comet assay kit (CA) and Annexin V-Fluorescein Staining kit (AV), we investigated the specific periods of appearance of apoptosis signals to clarify the relationship between apoptosis and embryogenesis in sea urchins. The CA is a method of detection for DNA fragmentation at apoptosis, which induces comet-like tails. Using the CA, we found long tails in the whole embryo and the dissociated cells from the embryo at the swimming blastula stage. No clear tails were found at the mid-gastrula stage. The clear tails were observed again in the late gastrula stage. AV detects phosphatidylseline which is translocated from the inner to the outer cell membranes by apoptosis in its early stage. We also found a clear-colored reaction to AV in the embryo at the swimming blastula and late gastrula stages. At the mid-gastrula stage, it was difficult to observe the reaction of AV. We found the clear-colored reaction to AV prior to the appearance of long clear tails by the CA. Taking together these results, it seems that there are two peaks of appearance of apoptosis signals at the blastula and gastrula stages. These signals may play a role in the morphogenesis in sea urchin embryos.

LOCALIZATION OF SYNDECAN IN EMBRYOS OF THE SEA URCHIN *ANTHOCIDARIS CRASSISPINA*

Isao Uemura[1], Akie Nitta[2], Kazuo Tomita[2], Kyo Yamasu[2], Takashi Suyemitsu[2]
[1]Department of Biology, Faculty of Science, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan and [2]Department of Regulation Biology, Faculty of Science, Saitama University, Saitama, Saitama 338-8570, Japan

When embryos of the sea urchin *Anthocidaris crassispina* were cultured in the presence of the antibody against the sea urchin syndecan, severe inhibition of elongation of the postoral arms was seen in treated embryos. Moreover, in the treated embryos, cell number continued to increase normally until 31 hpf, while cell division stopped after 31 hpf. In the present study, in order to reveal the biological role of the sea urchin syndecan, localization of syndecan was examined in embryos and dissociated cells of *Anthocidaris crassispina*, using light and electron microscopic immunohistochemistry. Light microscopic observation revealed a fluorescent dot of considerable size in each ectoderm cell of whole-mounted late gastrulae. These dots were found between the nuclei and the apical surface with simultaneous staining of nuclei, implying that they are the Golgi apparatus. Hyaline layer was also stained. In dissociated cells, similar dots were observed in close vicinity of nuclei in each cell. Electron microscopic observation detected possible signals on Golgi vesicles, acidic vesicles, and hyaline layer.

DO PIGMENT CELLS EXERT THE MOTIVE FORCE FOR GASTRULATION IN ECHINOIDEA?

Hiromi Takata, Tetsuya Kominami
Dept. Biology and Earth Science, Faculty Science, Ehime Univ., Ehime, Matsuyama 790-8577, Japan

In sea urchin embryos, secondary mesenchyme cells (SMCs) have been suggested to play an important role in gastrulation. However, it is still unclear which type of SMCs exerts the force for gastrulation. To know whether pigment cells play a role in gastrulation, we observed gastrulating embryos of four regular and two irregular echinoids. In *E. mathaei* and *M. globulus*, the manner of gastrulation was almost the same as that observed in *H. pulcherrimus* (stepwise), while the manner in *T. pileolus, C. japonicus* and *A. manni* was similar to that in *S. mirabilis* (continuous). In the embryos that showed a typical pattern of gastrulation, pigment cells were observed mainly at the archenteron tip during gastrulation. On the other hand, pigment cells had already dispersed in the ectoderm before the onset of gastrulation in the embryos that showed continuous invagination. In *A. crassispina*, the manner of gastrulation was not clear, and pigment cells were observed at both the archenteron tip and vegetal ectoderm. Thus, the behavior of pigment cells did not strictly but closely correlate to the manner of gastrulation in Echinoidea.

FUNCTIONAL ANALYSIS OF EXTRACELLULAR ARYLSULFATASE

Keiko Mitsunaga-Nakatsubo, Eiji Akiba, Hiraku Shimada, Koji Akasaka
Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8526, Japan

We have demonstrated that arylsulfatase (HpArs) functions as a non-enzymatic cell surface protein and is involved in the process of epithelial folding for gastrulation and arm rudiment formation during the development of the sea urchin, *Hemicentrotus pulcherrimus*. Morphological normal phenotypes in the embryos injected with *HpArs* mRNA suggests that the interaction with another extracellular matrix (ECM) proteins needs for HpArs function. In order to gain insight into the role of HpArs during morphogenesis, the interactions between HpArs and ECM proteins were analyzed. HpArs accumulation was markedly reduced in the embryos cultured with β-aminopropionitrile, an inhibitor of collagen cross linkage. Purified HpArs protein showed the high affinity with heparin and the loss of enzymatic activity in the presence of Ca^{2+} and Mg^{2+}, *in vitro*. These results suggest that the HpArs secreted into the ECM on the apical surface of the aboral ectoderm binds specifically to proteoglycan and directly or indirectly to collagen, and is involved in the morphogenetic movements during sea urchin development.

FORMATION OF SEROTONIN RECEPTOR CELL NETWORK IN SEA URCHIN LARVAE

Hideki Katow, Kei-ichiro Kyozuka, Ritsu Kuraishi
Marine Biological Station, Graduate School of Science, University of Tohoku (Tohoku University), Asamushi, Aomori, Aomori 039-3501, Japan

Serotonergic neurons comprise the first nervous system formed during early embryogenesis in sea urchin. However, its target cells, and how the neurotransmitter works have long been left unanswered. In this study we have cloned a fragment of serotonin receptor protein (5-HTR) from a sea urchin cDNA library, synthesized a peptide based on the sequence that encodes hydrophilic region of 5-HTR, and used for raising antibody. The antibody recognized a 58kDa band protein that was first expressed at prism stage, and detected with maximum intensity in plutei. Epitope of the antibody was localized on the cytoplasmic side of the plasma membrane of blastocoelic cells. These cells formed a multicellular network that was comprised of 6 sub-groups of cells that distributed along the antero-posterior axis of pluteus on both left and right sides of ventro-posterior region (in trunk) and ventro-anterior region (in oral lobe), and these 4 sub-groups were connected with a transverse fibers on the dorsal side at esophagus region. An additional dorsal fiber was extended from the dorsal esophagus region to posterior trunk. All serotonin-applied 5-HTR cells by microinjection released Ca^{2+}.

FORMATION OF SEROTONERGIC APICAL GANGLION AND POSSIBLE ROLE OF SEROTONIN IN SWIMMING BEHAVIOR AND MORPHOGENESIS IN SEA URCHIN LARVAE

Shunsuke Yaguchi, Hideki Katow
Developmental and Cell Biology (Marine Biological Station), Graduate School of Life Sciences, Tohoku University, Asamushi, Aomori, Aomori 039-3501, Japan

Tryptophan 5-hydroxylase (TPH) message transcription site was examined in plutei of the sea urchin, Hemicentrotus pulcherrimus, using TSA technique along with the influence of *p*-chlorophenylalanine (CPA), a specific inhibitor of TPH, to differentiation of serotonin cells. Possible role of serotonin also was examined using CPA.