Lineage and induction of epidermal sensory neurons of ascidian larvae

Yukio Ohtsuka[1], Jun Matsumoto[1], and Yasushi Okamura[2].

[1]Neuroscience Research Institute, AIST, [2]Center for Integrative Bioscience, Okazaki National Research Institutes.

Epidermal sensory neuron (ESN) is a peripheral neuron in ascidian larvae. We previously reported that in Halocynthia embryos, ESNs lie in the dorsal epidermis of the head, neck, and tail regions. In this study, we examined the lineage and specification of ESNs by using gelsolin, as a suitable molecular marker for ESNs. To investigate the lineage of ESNs, immunostaining with anti-gelsolin antibody was performed on the embryos in which the plasmid, containing LacZ gene linked to the neuron-specific promoter, was injected into a4.2 or b4.2 blastomere. We found that ESNs in the head region are derived from a-line cells and that ESNs in the neck and tail regions originate from b-line cells. In addition, we showed that ESNs developed when either a-line cells or b-line cells were treated with bFGF. However, ESNs were strongly induced in treatment of ectodermal cells (a4.2+b4.2) with bFGF, suggesting that the cell-cell interaction between a-line cells and b-line cells plays an important role in the induction of ESNs by bFGF.

Roles of Ca^{2+}, Golgi transport and suger residue on the cell adhesion of blastomeres in the embryo of the ascidian, Phallusia nigra

N. Sensui[1] and M. Morisawa[1].

[1]Anatomy 1, Faculty of Med., Univ. of the Ryukyus, Okinawa; and [2]Misaki Marine Biological Station, Grad. Sch. of Sci., Univ. of Tokyo, Miura.

Adhesion between blastomeres of the embryo is necessary for morphogenesis during the development. We have shown that eggs of ascidian, *Phallusia nigra* whose the vitelline layers are removed can acquire the capability to adhere each other after completion of meiosis by fertilization. We showed here that the adhesion of the fertilized egg and blastomeres required extracellular Ca^{2+}. The capacity for the adhesion in the cells was reduced by brefeldin-A, an inhibitor of trans-Golgi transport, and the goat serum that contains a component for binding to the surface of egg and blastomeres. Topological position of blastomeres in an 4-cell embryo was perturbed by treatment of the fertilized egg with brefeldin-A and Ca^{2+} free medium. These results suggest that molecule(s) responsible for the Ca^{2+}-dependent cell adhesion is transported through Golgi apparatus and appears on the surface of the egg. We further showed that WGA and GSII, which recognize GlcNAc were bound to the surface of egg and embryo. However, the binding was not changed when the egg or blastomeres acquired the adhesiveness, suggesting that sugars are not the factor indispensable for the adhesion of the blastomeres.

Studies on the neural-tube specific gene of the ascidian larva

K. Etani[1] and T. Nishikata[2,3].

[1]Grad. Sch. Natural Sci., Konan Univ., [2]Fac., Sci., Eng., Konan Univ., [3]HRC, Konan Univ., Kobe.

Neural tube is one of the characteristic features of chordates. Ascidian neural tube consists of about 330 cells and has relatively simple architecture. In order to understand how vertebrates evolved from invertebrates, it is very important to reveal the molecular mechanisms, which underlying the ascidian neural tube development.

We have cloned a newly identified gene, designated as a28, which expressed specifically in the entire neural tube of the ascidian *Ciona intestinalis*. When compared with amino acid sequences in databases, a28 showed weak homology to the known neural receptors, such as adrenalin receptor an rhodopsin. Moreover, seven transmembrane domains are predicted in the amino acid sequence of a28. According to these results, a28 have a possibility to have an important role for the signal transduction during the neural tube formation in the ascidian development as a G-protein coupled receptor. So we tried to ascertain the a28 expressing blastomeres precisely by using the *in situ* hybridization and nuclear staining. Thereby, we could estimate the function of the a28 during ascidian neural tube formation.

Analysis of expression of *Hrpitx*, an ascidian homologue of vertebrate *pitx* genes, in the development of *Halocynthia roretzi*

M. Ueno and H. Saiga.

Dept. Biol. Sci., Grad. Sch. Sci., Tokyo Metropolitan Univ., Tokyo

Ascidians, a member of lower chordates, have been thought to be close to an ancestor of chordates and exhibit simple but similar morphogenesis to that of vertebrates. Thus, ascidians serve as a good model system for studying a basic morphogenetic mechanism in chordate embryos. Left-right asymmetry is one of the features in the development of vertebrates. In ascidian development, morphogenetic asymmetry is first observed at the tailbud stage, in which the trunk rotates clock-wise against the tail. In vertebrates, it has recently been reported that *pitx2* is expressed in the lateral plate mesoderm, which involves in asymmetric development of internal organs. In the present study, we have isolated an ascidian homologue of *pitx2* from *Halocynthia roretzi* and examined its expression during development. We found that the ascidian *pitx2* homologue was expressed in the palp lineage in the neurula, in the neurohypophysis and left epidermis at the tailbud stage and in a part of the sensory vesicle at the mid to late tailbud stages. This expression pattern is quite reminiscent of vertebrate *pitx1*, *2* and *3*, and the left-sided expression of *pitx* is conserved in the ascidian development.

Isolation and characterization of a DSL family gene of the ascidian *Halocynthia roretzi*

T. Akanuma, S. Hori, H. Nishida.

Dept. of Biol. Sci., Tokyo Inst. of Tech., Yokohama

In many animals, Notch signaling plays important roles in cell-cell interactions, especially in inhibitory interactions within neuroectoderm. Previously, we reported that Notch signaling functions during ascidian neurogenesis. The overexpression of active forms of HrNotch leads to suppression of adhesive organ and peripheral nervous system formations, and promotion of epidermal differentiation instead. This suggests that Notch signaling is involved in fate choice between adhesive organs and epidermis and between peripheral neurons and epidermis.Here we report isolation and characterization of a DSL family gene of *Halocynthia roretzi*, which would act as a ligand in Notch signaling pathway. The broad expression domains were gradually restricted to the nervous system as embryogenesis proceeded. At the late tailbud stage, the expression was observed in adhesive organs and peripheral neurons. These results further support the idea that Notch signaling determines the fate choice between neurons and epidermis during ascidian neurogenesis.

EXPRESSION PATTERNS OF THREE BRAIN VESICLE-SPECIFIC GENES DURING EMBRYOGENESIS OF THE ASCIDIAN *CIONA INTESTINALIS*

R. Kusakabe[1], T. Kusakabe[1], I. Kawakami[1], Y. Satou[2], N. Satoh[2] and M. Tsuda[1].

[1]Dept. Life Sci., Himeji Inst. Tech., Hyogo and [2]Dept. Zool., Grad. Sch. Sci., Kyoto Univ., Kyoto.

The brain of the ascidian larva contains a photoreceptor organ (ocellus) and a gravity sense organ (otolith). Since neuronal differentiation markers have not been available, previous studies on the sensory organ development only focused on pigment cell differentiation. Here we report primary structure and expression patterns of mRNAs encoding sensory signal transduction proteins of the ascidian *Ciona intestinalis*. A homologue of vertebrate opsin gene, *Ci-opsin1*, is expressed in photoreceptor cells of the ocellus. *Ci-PDEd1* encodes a cGMP phosphodiesterase delta subunit. It is not expressed in the ocellus but in the cells neighboring the pigment cell of the otolith. *Ci-RGR1* encodes a G protein-coupled receptor similar to mammalian retinal protein RGR. *Ci-RGR1* is expressed in the brain vesicle and motor neurons. The expression of *Ci-RGR1* begins at the neural plate stage, while transcripts of *Ci-opsin1* and *Ci-PDEd1* first appear at early and mid tail bud stages, respectively. We will discuss cell lineages and cell-specification mechanisms of nerve cells of the otolith and ocellus based on the expression patterns of *Ci-opsin1* and *Ci-PDEd1*.