DIFFERENTIATION OF SEROTONERGIC NERVE CELLS IN THE PARTIAL EMBRYOS OF SEA URCHINS

S. Amemiya[1] and Y. Nakajima[2]. [1]Dept. of Integ. Sci., Grad. School. of Front. Sci., Univ. of Tokyo, Tokyo, [2]Dept. of Biol., Keio Univ., Yokohama.

In normal development of sea urchins, 16-cell-stage embryos are composed of eight mesomeres (animal cap), four macromeres and four micromeres from an animal to vegetal pole direction. The animal cap in the undisturbed normal embryos differentiate exclusively into ectoderm. The macromeres contain three different presumptive germ layers, ectoderm, endoderm and mesoderm. The micromeres differentiate into two mesodermal cell typoes, skeletogenic mesenchyme derived from large micromeres and coelomic pouch constituents derived from small micromeres. The serotonergic nerve cells in the sea urchin larvae had been reported to originate from the cells around the animal pole derived from the mesomeres. In the present study, the developmental potential of the partial embryos produced by dissecting the 16-cell-stage embryos at equatorial plane to differntiate the serotonergic nerve cells was examined. All embryos derived from the animal cap developed into permanent blasturae, and a considerable fraction of the embryos derived from the vegetal half composed of four macromeres and four micromeres developed into apparently normal pluteus. The total number of the serotonergic nerve cells differentiated in the embryos and larvae derived from the partial embryos was almost twice of undisturved normal larvae. The results suggest that signals from the vegetal half inhibit differentiation of the serotonergic nerve cells in the animal cap of the normal embryos.

THE CHANGES AND DISTRIBUTION OF CYCLIN B1 DURING OOCYTE AND EARLY EMBRYONIC CELL CYCLE IN NEWT.

T. Murakawa[1], H. Abe[1], R. Murakami[1], Y. Iwao[1] and M. Yamashita[2]. [1]Dept. of Biol. Sci., Fac. of Sci., Yamaguchi Univ., Yamaguchi, [2]Div. of Biol. Sci., Graduate School of Sci., Hokkaido Univ., Hokkaido.

The entry into M phase in sperm nuclei is regulated by the differential distribution of maturation-promoting factor (MPF) in phygiologically polyspermic newt, *Cynops pyrrhogaster*, eggs. In order to know MPF distribution during oocyte maturation and early embryonic cell cycle , we examined the changes and distribution of the cyclin B1 proteins and mRNAs. In both unfertilized and fertilized eggs, the amount of cyclin B1 in animal hemispheres was larger than that in vegetal hemispheres. Not only the cycling in the amount of cyclin B1, but also the shift in its mobility on SDS-PAGE was observed during early embryonic cell cycles, indicated the different phosphorylation of cyclin B1. In addition, we have tried to show the distribution of the cyclin B1 mRNAs in the oocytes. The cyclin B1 mRNAs was abundant in cytoplasm, but was not found in germinal vesicles and in follicle cells.

CELL FATE CHANGE BY CYTOPLASMIC TRANSFER TO BLASTMERES OF THE ASCIDIAN, *Halocynthia roretzi*.

— Transfer of cytoplasm containing endoderm determinants —

Kenji Kobayashi and Hiroki Nishida.
Faculty of Biosci. and Biotech., Tokyo Inst. of Tech., Yokohama.

Endoderm, muscle and epidermis differentiate cell-autonomously by inhering localized ooplasmic determinants during ascidian embryogenesis. Some of genes specific to such tissues starts their expression at cleavage stage.

In this study, we transferred cytoplasm containing endoderm determinants to presumptive-muscle and presumptive-epidermis blastmeres isolated from late-cleavage stage embryos in which the tissue-specific genes are already expressed, and then examined whether ectopic endoderm formation (i.e. cell fate change) occurs. The results showed that cell fate can be changed by cytoplasmic transfer, even in the recipient blastmeres in which the cell fates are already restricted only one cell type as well as in those where expression of the tissue-specific genes are already initiated. Muscle or epidermis tissue-specific genes that are already expressed in the recipient blastmere were down-regulated in ectopically forming endoderm cells.

Regulation of somatic cell plasticity in budding tunicates. I. Serine protease induces dedifferentiation of multipotent epithelium.

K. Kawamura, S. Fujiwara, N. Fujii, M. Oohashi#, and T. Yubisui (Lab. of Mol. Cell. Biotech., Fac. of Sci., Kochi Univ., #Div. of Biol. Sci., Nagoya Univ.)

It is well known that serine proteases exhibit a variety of functions in animal fertilization, body axis formation, cell growth, and tumor metastasis. In the budding tunicate, *Polyandrocarpa misakiensis*, trypsin-like activity is induced by retinoic acid (RA) that is a primary inducer of morphallactic regeneration of buds. It brings about proliferation and dedifferentiation of multipotent cells, the atrial epithelium in vitro.

We have recently isolated a cDNA of serine protease homolog (TRAMP, tunicate retinoic acid-inducible modular protease), of which the deduced open reading frame is of 2.8 kb. It consists of N-terminal multifunctional domain such as low density lipoprotein receptor and C-terminal catalytic domain. Recombinant C-terminal TRAMP showed both trypsin-like and thrombin-like protease activity. It promoted in vitro cell proliferation and cell motility of cultured multipotent epithelium. These activity could not be disturbed by site-directed mutagenesis of proteolytic active sites, suggesting that, like urokinase-type plasminogen activator, a proteolytically inactive form of TRAMP is able to induce mitogenic response in serum-deprived cells. Both results of in situ hybridization and immunohistochemistry showed that TRAMP is expressed by specific kind(s) of mesenchymal cells. Anti-TRAMP monoclonal antibody also showed that RA-inducible trypsin like activity mentioned above contains TRAMP immunoreactivity.

We are now examining whether TRAMP is able to trigger the ectopic morphogenesis of tunicate buds. The result will afford direct evidence for TRAMP as driving force of animal regeneration.

ASCIDIAN PRIMORDIAL GERM CELLS EXIST IN THE LARVAL TAIL.

M. Fujimura[1], K. Takamura[2] and Y. Yamaguchi[1]. Dep. of Biotech.[1]; and Dep. of Marine Biotech.[2], Fac. of Engineer., Fukuyama Univ., Fukuyama.

To investigate the origin of germ cells in ascidian, *Ciona intestinalis* and *Ciona savigni*, we isolated ascidian *vasa* homologues (*Ci-vasa* and *Cs-vasa*) from ovary cDNA libraries by polymerase chain reaction. *Ci-vasa* cDNA clone was inserted into pGEX-4T vector and GST/Ci-vasa-fusion protein was synthesized *in vivo* in host *E. coli* transformed by it. This fusion protein was purified through gulutation-sepharose column and used as immunogen for monoclonal antibody preparation. We performed immunohistostaining with larvae and juveniles during and after metamorphosis using obtained anti-Ci-vasa monoclonal antibody. In larvae, this antibody stained 8 and 4 cells in the caudal endodermal strand of *C. intesinalis* and *C. savignyi*, respectively. These stained cells moved into the posterior part of the trunk during tail absorption, and then invaded into the gonad rudiment as juveniles growing. Moreover, in the juveniles from the tail-cutted larvae, no anti-Ci-vasa-positive cells were found in their gonad rudiment. These results show clearly that the origin of asidian primordial germ cells is a part of the caudal endodermal strand of larvae.

ROLE OF SPARC IN MYOGENESIS

°M.SHIOZUKA[1,2], R.MATSUDA[2], and I.KIMURA[3]
[1]Advanced Research Center for Human Sci., Waseda Univ.
[2]Dept. of Life Sci., Graduate Sch. of Arts and Sci., Univ. of Tokyo
[3]Dept. of Basic Human Sci., Sch. of Human Sci., Waseda Univ.

Our previous studies have demonstrated that follistatin enhanced myogenic differentiation in culture. This stimulatory effect was also elicided by its related molecules such as SPARC (Secreted Protein-Acidic and Rich in Cysteine, also known as osteonectin or BM-40).

In this study, we focus on the bioavailability of SPARC and its interaction with other growth factors during myogenesis. Application of exogeneous SPARC to chick myogenic cell cultures resulted in the counteraction to myogenesis-inhibiting effect of activin A and exerted an influence similar to follistatin. In addition, the spatiotemporal distribution of SPARC protein in the developing chick embryo was examined by whole-mount immunohistochemical techniques. These results suggest possible role of this protein as a potent myogenic regulator.