Universal occurence of the *vasa* (*vas*) related genes in matazoans

K. Mochizuki, C. Nishimiya-Fujisawa and T. Fujisawa,

Department of Developmental Genetics, National Institute of Genetics, Mishima 411-8540, Japan.

The *vas* related genes have been reported only in metazoans and shown to be expressed specifically in germline cells in all the animals so far examined. To investigate the origin of the *vas* related genes and the time of acquiring the germline specificity during evolution, we have analyzed the *vas/PL10* class genes in lower metazoans like sponge, hydra, jellyfish and planaria.

Using RT-PCR, we obtained cDNAs respectively encoding the *vas* and *PL10* related proteins from a sponge (*Povas1* and *PoPL10*), hydra (*Cnvas1*(Hm), *Cnvas2*(Hm) and *CnPL10*(Hm)), a jellyfish (*Cnvas1*(Aa), *Cnvas2*(Aa) and *CnPL10*(Aa)) and a planaria (*Plvas1*, *PlvlgA* and *PlvlgB*). Thus, the *vas* related genes already existed in Porifera. The phylogenetic analysis suggests that the *vas* related genes arose by duplication of the *PL10* related genes before the appearance of sponges but after the diversion of fungi and plants.

Expresion patterns of the mRNAs of the *vas* and *PL10* related genes were examined in hydra. *Cnvas1* and *Cnvas2* showed the almost identical expression patterns. They are mainly expressed in germ cells and multipotent stem cells which give rise to both germ cells and somatic cells (nerves, nematocytes and gland cells). The expression halts immediately after the multipotent stem cells enter the somatic pathway. Weak expression was also observed in the ectodermal epthelial cells of the body column. In contrast, *CnPL10* appears to be expressed both in germline cells and somtatic cells in the interstitial cell lineage. These results suggest that both the occurence and germline specificity of the *vas* related genes were established before the Cnidarian evolution.

BRACHYURY (T)* GENE AND NOTOCHORD DEVELOPMENT OF THE APPENDICULARIAN, *OIKOPLEURA LONGICAUDA

Atsuo Nishino[1], Yutaka Satou[1], Masaaki Morisawa[2], Nori Satoh[1].

[1]Dept. of Zool., Grad. Sch. of Sci., Kyoto Univ., Kyoto. [2]Misaki Marine Biological Station, Univ. of Tokyo, Miura.

Appendicularians retain their tail throughout their pelagic life. Information has recently accumulated that indicates a significance of appendicularians to interpret original features of chordates, but detailed analyses on their development have been limited. Since notochord is one of the most outstanding features of chordates, it is important to define characteristics of notochord in appendicularians. We isolated from the appendicularian, *O. longicauda*, genomic clones encoding *Brachyury (T)* gene that is a crucial player for notochord development in other chordate groups. Phylogenetic analysis of the T-box coding sequence positioned it within the *Brachyury (T)* subfamily. The genomic clone contains two introns within its T-box, whose positions do not correspond to any intron positions conserved among T-box family genes of other organisms. The entire sequence of the cDNA was also determined, and the expression patterns were examined by whole-mount in situ hybridization. This revealed its constitutive expression in the notochordal cells from gastrula to late infant stages. Transient expression was also found in a few cells of the infant trunk. We also present general descriptions on the process of notochord formation as well as more detailed analyses on the structures of the genomic sequence.

OCCURRENCE OF PLACOZOANS IN SHIRAHAMA.

Yoshihiko K. Maruyama
Section of Marine Bio. Sci., Edu. & Research Center for Bio. Resources, Fac. of Life & Environ. Sci., Shimane Univ., Shimane

Placozoans are an unusual basal metazoan with a ciliated plate-like morphology. It has been thought that it may be a model for an early metazoan ancestor. Results of this study show that placozoans occur nearly throughout the year at a subtidal site. The late summer or autumn appeared to be rich in placozoans. It is also shown that they occurred every year for ten years. Specimens thus obtained could be reared on films of food materials prepared on clean glass slides, where they reproduced asexually. The laboratory rearing for one year or more at 20°C was possible. In the laboratory rearing, the appearance of placozoans with two-cell stage embryos was confirmed.

Occurrence of placozoans in a sea water aquarium was also reported.

DEVELOPMENT OF THE SEA STAR, *APHELASTERIAS JAPONICA*.

M. Komatsu, H. Yamanishi. Dept. of Biol., Fac. of Sci., Toyama Univ., Toyama.

Development through metamorphosis of the sea star *Aphelasterias japonica*, was observed. The breeding season in Mutsu Bay, Morioka Pref., occurs in October through the biginning of November. Eggs are approximately 135 μm in diameter. The first cleavage occurs 3 hr after fertilization at 15°C. Cleavage is total, radial and equal. Twenty hr after fertilization hatching takes place. The embryo develops into a bipinnaria after a wrinkled blastula stage. Three and one half days after fertilization, early bipinnariae have a left posterior coelomic vesicle at the left side of their stomach. Fifteen days after fertilization the tips of the right and left coelomic pouches are in close contact with each other. At this stage, spicules, corresponding to the rudiments of terminal plates appear on the posterior part of the bipinnnaria. One mo after fertilization, bipinnariae are transformed into a brachiolaria. The full-grown brachiolaria is 2.5 mm long.The bipinnaria arms are by now well developed; in particular, one pair of the lateral arms were 2 mm long, Organs of attachment are three brachiolar arms and a sucker among them. Brachiolar arms are covered with papillae at their end. Metamorphosis takes place 3 mo after fertilization. At metamorphosis the larval part is absorbed into the asteroid rudiment. Newly metamorphosed juveniles are 900 μm in diameter. They have 5 arms, each bearing 2 pairs of tube-feet and a terminal tentacle in each arm. The present observation show that *A. japonica* undergoes an indirect type of development.

A cDNA project of *Ciona intestinalis* (II)

Naohito Takatori, Akane Sasaki, Makoto Hamaguchi, Hitoe Metoki, Yasuaki Mochiduki, Rikishi Yamada, Yutaka Satou and Noriyuki Satoh

Department of Zoology, Graduate School of Science, Kyoto University.

As a part of the *Ciona intestinalis* genome project, we have been working on cDNA project. First, we constructed an arrayed cDNA library of the tailbud-stage embryo. Then the 3'end of each clone was sequenced to determine the independency of the clones. The 5'end sequence was also determined to infer the similarity to reported cDNAs. Finally, by whole-mount in situ hybridization, we are making clear the comprehensive expression pattern of the mRNAs. We here report the progress.

HATCHING ENZYME-LIKE MOLECULE IN PREHATCHING QUAIL EMBRYO

Shigeki Yasumasu[1] and Norio Yoshizaki[2]

[1]Life Sci. Inst., Sophia Univ., Tokyo, [2]Dept. of Agriculture, Gifu Univ., Gifu.

Avian hatching process involves a series of complicated phases including the digestion or breakdown of vitelline membrane, egg white, shell membrane and eggshell. Participation of hatching enzyme-like molecule at the first phase, digestion of vitelline membrane, has been suggested from existence of a molecule(s) that cross-reacts to a polyclonal antibody against *Xenopus* hatching enzyme (Yoshizaki, 1999). To clone cDNA of quail hatching enzyme, a degenerate primer set was constructed from amino acid sequences conserved in fish and frog hatching enzymes, and the cDNA was amplified by RT-PCR and RACE method. The cDNA thus cloned was 1,516bp-long, contained an open reading frame encoding 405 amino acids, and was tentatively named Qhe. Sequence analysis suggested that the Qhe was synthesized as a preproenzyme, and the mature enzyme comprised an astacin-like protease domain and a CUB domain. The astacin-like protease domain was highly homologous to that of known hatching enzymes (52% for Xhe, 49% for HCE). Molecular phylogenetic analysis on an astacin family consisting of astacin, hatching enzyme, BMP, tolloid and meprin revealed that the Qhe belonged to the hatchig enzyme group. By northern blot analysis, the transcript of Qhe was first found in day-0 embryo, and increased in amount until day-2. After that, the expression was gradually decreased, and kept a low level. In day-8 and day-9 embryos, the transcript was dramatically increased, and the strongest expression was observed in day-9 and day-10 embryos. Thereafter, the transcript was gradually diminished. Thus, the expression profile of Qhe gene was biphasic. The first phase of the expression occurred just before vitelline membrane digestion. In addition, the results suggest that the Qhe plays another role at the later stage of development.