CLONING OF A QUAIL HOMOLOGUE OF HATCHING ENZYME AND ITS EXPRESSION

○Norio Yoshizaki[1], Shigeki Yasumasu[2]
[1]Department of Animal Science, Faculty of Applied Biological Science, Gifu University, Gifu 501-1193, Japan, [2]Institute of Life Science, Sophia University, Chiyoda-ku, Tokyo 102-0094, Japan

We isolated a 1510-bp cDNA from extraembryonic tissues of Japanese quail *Coturnix japonica* embryos and designated it quail hatching enzyme (QHE) cDNA. The QHE cDNA was found to code a protein molecule comprising an astacin protease domain in the N-terminal half and a CUB domain in the C-terminal half. A phylogenetic analysis showed that QHE belonged to the hatching enzyme group and was distinct from other proteases in the astacin family. Northern blotting and in situ hybridization demonstrated that expression of the QHE mRNA occurred twice during the development of quail embryos; first in ectodermal cells of the yolk sac on Days 0-5, then in those of the albumen sac on Days 8-13. Zymography revealed that proteolytic activity in extracts of Days 3-4 and 9-12 embryos appeared in a protein band at the position corresponding to a molecular mass of 40 kDa. Immunoblotting tests showed that anti-QHE antiserum stained a 40-kDa molecule in extracts of Day 3 area vitellina. Anti-QHE antibody stained the ectodermal cells of the area opaca on Days 0-1, those of the area vitellina of the yolk sac on Days 2-5, and those of the albumen sac on Days 9-12.

DEVELOPMENT OF DUAL-REPORTER TRANSGENIC MICE FOR READTHROUGH ASSAY

○Masataka Shiozuka, Kenichi Shimada, Ryoichi Matsuda
Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

We previously reported that negamycin causes readhrough of premature termination codons (PTC) and rescues dystrophin expression in *mdx* skeletal and cardiac muscles with less side effects than gentamicin. We suggest that negamycin is a therapeutic candidate for not only *Duchenne* muscular dystrophy but also other genetic disorders caused by nonsense mutations. In order to investigate more potent readthrough stimulator without toxicity and facilitate studies, we have developed a new *in vivo* dual-reporter assay system for measuring readthrough efficiency. Dual-reporter gene is composed with *beta-galactosidase* gene and *luciferase* gene connected with PTC driven by *CMV/beta-actin* hybrid promoter, and allows a simultaneous sensitivity and quantitative accuracy. We have established three transgenic mouse strains containing different PTC (TAA, TAG, TGA). Using these transgenic mice, we succeeded in the evaluation of readthrough activity in each tissue *in vivo* and in real time *in vitro* for the first time. The chemotherapeutic agents searched for the ability to readthrough, may form the basis of the effective therapy for inherited diseases involving nonsense mutations.

POSITION ADJUSTMENT OF ZEBRAFISH CaP PRIMARY MOTONEURON IN SPINAL CORD MAY BE INFLUENCED BY Sema3A2

○Mika Sato-Maeda, Masuo Obinata, Wataru Shouji
Department of Cell Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-8575, Japan

In zebrafish embryos, each somitic hemi-segment is typically innervated by three identifiable primary motor neurons in spinal cord: CaP, MiP, and RoP. They exit spinal cord from a single exit-point and at first extend ventrally on the medial surface of the somite. Previously we have reported that *nrp1a*, which encodes a component of Sema3A receptor, is specifically expressed by CaP during the early portion of its outgrowth. It was shown that antisense knockdown of *nrp1a* frequently causes double-exit phenotype in primary motoneurons at 22 hr embryos. Such phenotype was also induced by antisense knockdown of Sema3A2, while it was rarely observed in *sema3A1* morphants. In the antisense knockdown of *nrp1a*, the positions of CaP cell bodies were sometimes dislocated in relation to *sema3A2* expression pattern. These results suggest Sema3A2 involves the position adjustment of primary motoneurons.

VIDEO MICROSCOPICAL OBSERVATION ON THE STRUCTURE AND CONTRACTILE ACTIVITIES OF IMPERFECTLY DEVELOPED HEART OCCURRED IN THE CAUTERIZED *ORYZIAS* EMBRYOS

○Hajime Mori, Isao Uemura
Department of Biology Graduate School of Science, Tokyo Metropolitan University, Hachioji-shi, Tokyo 192-0397, Japan

We observed videomicroscopically structure and contractile activities of heart occurred in the damaged *Oryzias* embryos after topical application of microcauterizer at the anterior tip of the embryo. It seeems that damgae given to the prospective head region of *Oryzias* embryos at the earlier stages, which resulted in abnormal or imperfect head development, was not critical to tubular atrium formation. Tubular structure was also develped even when the anterior part of the atrium was mnissing, and its anterior end was closed. Heartbeats were usually observed in the imperfectly developed tubular atrium, and peristalsis was confirmed. Heart with two crescent-shaped atria was also observed in several cauterized embryos; contractile rhythms of two atria which developed in parallel with each other were not synchronous. Embryonic circulation was not occurred when atrial development was imperfect; this must be as due to lack of communication between atrium and vitelline vein. Heartbeats occurred in the imperfectly developed heart of cauterized Oryzias embryos were demonstrated by GIF computer animation processed from consecutive still images of video records.

CLONING OF HEMICENTROTUS PULCHERRIMUS DOPAMINE RECEPTOR D1

○Takayuki Jinnai, Masataka Nikaido, Takashi Suyemitsu
Department of Regulation Biology, Graduate School of Science and Engineering Master Couse, Saitama University, Sakura-ku, Saitama-shi, Saitama, 338-8570, Japan

It has been suggested that dopamine accelerates metamorphosis of sea urchin larvae. In this study, we tried to isolate a gene encoding the dopamine receptor D1 from the sea urchin H. pulcherrimus. First, we searched conserved amino acid regions in the dopamine receptor D1 of the sea urchin S. purpuratus and several vertebrates. We designed degenerate primers against these regions and performed PT-PCR using total RNA extracted from the competent larvae. As a result, we isolated 552 bp cDNA clone which presumably encoded a part of the transmembrane domains of dopamine receptor D1. Then, in order to identify the entire coding region, we performed inverse PCR using the genomic DNA from a digestive organ of adult sea urchin and obtained a fragment including the initiation codon and the stop codon. Furthermore, we succeeded to isolate the entire coding region (1308 bp) by PCR. Translated DNA sequence (436 amino acids) was analyzed with BLAST (NCBI) and was similar to several vertebrates dopamine receptor D1. It was observed that the sequence had conserved amino acids which are important for binding to dopamine. The results suggest that this gene encodes sea urchin dopamine receptor D1.

EFFECTS OF DEPRESSION OF MATERNAL PLASMA THYROID HORMONE LEVELS ON OREXIN-A-IMMUNOREACTIVE CELLS OF THE MALE OFFSPRING

○Motoko Matsuda[1], Ritsuko Katoh-Semba[2], Shougo Yasuda[3], Atsuo Nakayama[4]
[1]Department of Embryology, Institute for Developmental Research, Human Service Center, Kasugai 480-0932, Japan, [2]Department of Perinatology, Institute for Developmental Research, Human Service Center, Kasugai 480-0932, Japan, [3]Central Hospital, Institute for Developmental Research, Human Service Center, Kasugai 480-0932, Japan, [4]Department of Embryology, Institute for Developmental Research, Human Service Center, Kasugai 480-0932, Japan

Thyroid hormone (TH) is essential for embryonic brain development. We administrated propylthiouracil (PTU), a thyroid hormone synthesis inhibitor, to pregnant rats on 13.5 days gestation and the behavior of their offspring was analyzed by the open field test in successive 3 days. PTU induced depression of plasma T3 levels at 24 hours after the PTU-injection and the levels recovered at 48 hours after the injection in the pregnant rats. The male offspring of control dams decreased in ambulation and rearing as test-days go by. In contrast, the male offspring of dams injected PTU did not show the decrease in ambulation and rearing. In these rats orexin-A-immunoreactive cells were distributed in the lateral hypothalamic area and number of the cells was not different from controls. These results indicated that depression of maternal plasma TH levels on 13.5 days gestation did not disturb the production of orexin cells. However, the possibility remains that fetal hypothyroidism affects functional maturation of orexin cells.

TOWARD THE EXPERIMENTAL ANALYSIS OF PREDATOR-INDUCED POLYPHENISM IN *DAPHNIA PULEX*

○Yuka Naraki, Shin Tochinai
Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita10, Nishi 8 Kita-ku, Sapporo 060-0810, Japan

It is common in *Daphnia* species that a variety of defense responses are produced in the presence of predator-released chemical signals (kairomones). Among them, it is well known that *Daphnia pulex* generates small protuberances called neckteeth in the dorsal neck region in the presence of the predatory *Chaoborus* larvae. To address the developmental mechanism for neckteeth formation, we have collected and bred *D.pulex* and *Chaoborus* larvae from a temporary pool in the campus, and have succeeded in inducing the neckteeth in juvenile *D.pulex* in the absence of predators by culturing the embryos removeed from the brood chamber in medium containing *Chaoborus* larvae culture supernatant. We have also succeeded in determining the precise *Daphnia* developmental stages sensitive for *Chaoborus* kairomones. The overall results clearly indicated that some changes in developmental pathways can be induced in response to environmental conditions. We would like to discuss about the interaction between early developmental process and environment which might possibly be involved in the evolutionary process.

REGULATION OF METAMORPHOSIS OF THE CORAL *ACROPORA* IN RESPONSE TO ENVIRONMENTAL CUES

○Masayuki Hatta[1], Ayaka Horikoshi[2], Chie Sasaki[3]
[1]Marine and Coastal Biology Center, Ochanomizu University, Bunkyo-ku, Tokyo 112-8610, Japan, [2]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, [3]Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Larvae of the coral *Acropora* settle on undersea substrates and metamorphose after the planktonic life. Metamorphosis and settlement never undergo unless appropriate environmental cues on the substrates. We isolated bacteria from substrates, that induce metamorphosis. In parallel, we also isolated bacteria that inhibit hormone-induced meta-