FLUORESCENCE EMISSION IN SEA URCHIN EMBRYOS, LARVAE AND ADULTS

M. Wakabayashi[1], H. Tominaga[1], M. Komatsu[1], M. Iseki[2], M. Watanabe[2], S. Nakamura[3]
[1]Dept. Biol. and [3]Dept. Env. Biol. & Chem., Fac. Sci., Toyama Univ., Toyama 930-8555, Japan, [2]Natl. Inst. Basic Biol., Okazaki NRI, Okazaki 444-8585, Japan

We have found that strong fluorescence emission occurred in sea urchin larvae when irradiated with U (330-385 nm) or BV (400-440 nm) or B (450-480 nm) excitation light (EXL) under a fluorescence microscope. EXL irradiation first broke red granules in the pigment cells, releasing green or blue fluorescent substance(s) into the cytoplasm of the pigment cells, then the entire pigment cells emit strong fluorescence. With prolonged EXL irradiation, the pigment cell itself bursted, dispersing the fluorescent substance(s) into the body cavity or seawater. Cells that can emit such fluorescence first appeared at the late-blastula stage, proliferated with development, and changed into the red-pigmented cells. Similar fluorescence emission was observed in the embryos, larvae and adults of *Clypeaster japonicus*, *Hemicentrotus pulcherrimus*, *Anthocidaris crassispina*, *Pseudocentrous depressus*, *Tripneustes gratilla*, *Toxopneustes pileolus*, *Peronella japonica*, *Echinometra* sp. and *Astriclypeus manni* of which *C. japonicus* larvae displayed the strongest fluorescence.

SM50 TRANSCRIPTIONAL REGULATION MECHANISM AND THE EVOLUTION OF SPICULE IN ECHINODERM

Mioko Matsubara[1], Eiichi Shyoguchi[2], Noriyuki Satoh[2], Kouji Akasaka[3], Mieko Komatsu[1], Hiroshi Wada[4]
[1]Department of Biology, Faculty of Science, Toyama University, Toyama, Toyama 930-8555, Japan, [2]Department of Zoology, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan, [3]Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8526, Japan and [4]Seto Marine Biological Laboratory, Graduate School of Sciences, Kyoto University, 459 Shirahama, Wakayama 649-2211, Japan

Echinoderm larvae show remarkable morphological variation. Echinoid and ophiuroid show pluteus larvae with well developed skeleton. The larvae of crinoid and holothurian also have spicules, although they remain as small particles. Starfish larvae do not form any spicules. In echinoid larvae, Ets directly activate expression of a spicule matrix protein, SM50. Its expression is restricted to the skeletgenic primary mesenchyme cells. In this study, We asked whether absence of spicules in starfish larvae is due to lack of *Ets* expression in larval stage, or lack of gene interaction between Ets and SM50. We investigated expression of the Ets during embryogenesis of starfish, *Asterina pectinifera*. *Ap-Ets* expression is restricted to the vegetal region of blastula, archenteron of gastrula and mesenchymal cells of bipinnaria. We suggest *Ap-Ets* has the function as the gastrulation and differentiation of migrating mesodermal cells. We are now investigating involvement of *Ets* in ophiuroid larval skeletogenesis. These analyses will reveal evolutionary history of larval skeleton in echinoderm and its molecular evolutionary background.

LARVAL DEVELOPMENT AND GENETIC DETECTION OF ECHINODERM HYBRIDS USING RAPD-PCR TECHNIQUE

Takako Chimura, Mieko Komatsu, Yuji Yamazaki
Department of Biology, Faculty of Science, Toyama University, Toyama, Toyama 930-8555, Japan

Komatsu and Chimura (2001) reported that embryos from six hybrid crosses had metamorphosed into juveniles. These included the interclass cross between the sea urchin *Hemicentrotus pulcherrimus*, which has a pluteus larva, and the seastar *Asterias amurensis*, which has a bipinnaria larva followed by a brachiolaria. Crosses were also made between two congeneric species of seastars with different mode of development: *Astropecten scoparius*, with a feeding bipinnaria but no brachiolaria stage, and *A. latespinosus*, with a non-feeding barrel-shaped larva. RAPD analysis was used to determine whether mixing of genomes had occurred in the hybrids. Juveniles of congeneric crosses shared RAPD bands with both parents, but the cross-class hybrids showed only maternal bands. RAPD analysis is seen to be effective for the study of echinoderm hybrids. Further genetic and developmental studies will be necessary to determine which cross-class and congeneric hybrid embryos complete metamorphosis.

EFFECTS OF GRAVITY DURING AMPHIBIAN METAMORPHOSIS

Keiko Kashiwagi[1], Hideki Hanada[1], Hideo Kubo[2], Tadashi Shinkai[3], Hirotada Fujii[4], Masamichi Yamashita[5], Tomio Naito[6], Akihiko Kashiwagi[1]
[1]Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Higashihiroshima 739-8526, [2]Department of Membrane Biochemistry, Tokyo Metropolitan Institute of Medical Science, Tokyo 113-8613, [3]Department of Neurobiology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, [4]School of Health sciences, Sapporo Medical University, Sapporo 060-8556, [5]Institute of Space and Astronautical Science, Sagamihara, Kanagawa 229-8510, and [6]Department of Biological Science, Shimane University, Matsue, Shimane 690-8504, Japan

This study attempts to shed some light on the possible effects of high gravity envionments on developing tadpoles. *Rana rugosa* tadpoles raised in 5G were delayed in development 15 days after the beginning of treatment. As the days went by, their development became strikingly retarded, and finally all tadpoles failed to undergo metamorphosis. 10G-reared tadpoles were all retarded in development after day 15, and by day 45 roughly half the tadpoles had died. In the case of *Bombina orientalis*, there were no marked differences between 5G-tadpoles and untreated controls with respect to developmental rate and metamorphosis. At 10G *B. orientalis* tadpoles beginning treatment at stage X were retarded in development, and only 14% of them completed metamorphosis. For tadpoles beginning treatment at stage XII, however, a striking increase in the number of metamorphosed frogs was noted. *Xenopus laevis* tadpoles at 5G were all poorly deeveloped by day 20. They showed no subsquent growth and failed to metamorphose. 10G-tadpoles showed incomplete development after day 20, and by day 50 only half of the tadpoles survived.

GABAERGIC INHIBITORY INNERVATION OF THE LATERAL HEART IN THE EARTHWORM, *AMYNTHAS* SP.

Hanae Yasuno, Kiyoaki Kuwasawa
Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan

Anterograde peristaltic movements originate at the posterior end of the dorsal vessel to push hemolymph anteriorly. *Amynthas* sp. possesses four bilateral pairs of the lateral hearts (LHs) in the 10th to 13th body segments. Myogenic beats of the LHs convey hemolymph from the dorsal vessel and the supraintestinal vessel to the ventral vessel. One of nerves which emerge from the 3rd root of the segmental ganglion in each of the 10th to 13th body segments enters the muscular intersegmental septum. Some of branches extending from the nerve innervate the LH. Stimuli applied to the nerve induced acceleration and inhibition of the LH. The results suggest that the LH may receive dual innervation from the nerve. Inhibitory junction potentials (IJPs) were recorded from LH muscle cells in a one to one manner in response to stimuli applied to the nerve. A train of IJPs induced by stimuli at more than 1 Hz showed facilitation and summation. When GABA was applied to the LH, cardiac arrest occurred. A GABAergic antagonist, bicuculine blocked IJPs evoked by stimulation of the nerve. GABA may be a candidate neurotransmitter for inhibitory axons innnervating the LH.

PACEMAKER MECHANISMS OF THE HEARTBEAT IN THE ISOPOD CRUSTACEAN *PORCELLIO SCABER*

Satoshi Takano, Hiroshi Yamagishi
Institute of Biological Sciences, University of Tsukuba

The heart is regulated in situ by nerves and neurohormons from the central nervous system. However, we found that the heart isolated from adults of the land isopod *Porcellio scaber* exhibits changes in beat frequency by application of light. To analyze the responses of the isolated heart to light, we first examined the pacemaker mechanisms of the heartbeat in adult of *P. scaber* with electrophysioloigical methods. Being different from the results of a previous work (in *P. dilatatus*, Delaleu *et al.* 1972), the heart did not stop beating by application of tetrodotoxin. Moreover the results of current injection into the cardiac muscle revealed that, though the cardiac muscle has properties of endgenous oscillator, the adult heart is fundamentaly neurogenic with the cardiac ganglion acting as a primary pacemaker. This type of cardiac pacemaker system is very similar to that of the littoral isopod *Ligia exotica*. The results suggest that the light changes the beat frequency of the adult heart of *P. scaber* by affecting the cardiac ganglion.

IDENTIFICATION OF CARDIOREGULATORY NEURONS IN THE THORACIC GANGLION OF *BOMBYX MORI*

Kazuyuki Uchimura, Kiyoaki Kuwasawa, Makoto Kurokawa
Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan

The dorsal vessel (aorta and heart) is innervated by four pairs of nerves in *Bombyx mori*. One of the four pairs is a pair of branches of the dorsal nerve extending from the 1st abdominal ganglion. The branch may contain accelerator axons for the anterograde heartbeat (Ai and Kuwasawa 1995). No branch extended from the dorsal nerve to the dorsal vessel (alary nerve-1) in larvae and adults of *Manduca Sexta* (Davis *et al.* 2001). In this study, we adopted techniques of axonal back- and forward-filling to trace the nerve between the central nervous system and the dorsal vessel. The nerve to the dorsal vessel was back-filled with rhodamine-dextran at the proximal cut stump. We identified three neuronal somata in the 3rd thoracic ganglion. The nerve to the dorsal vessel was forward-filled with rhodamine-dextran at the distal cut stump. We found that the nerve bifurcated anteriorly and posteriorly on the dorsal vessel just at the site where the nerve entered the dorsal vessel. The branch running posteriorly may contain a cardioaccelerator axon which reaches the end of the heart. The function of the axon running anteriorly remains to be examined.

PHASE SHIFTS INDUCED BY LONG-DAY PULSES IN THE CIRCANNUAL PUPATION RHYTHM OF *ANTHRENUS VERBASCI*

Tomoyosi Nisimura, Hideharu Numata
Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan

The varied carpet beetle *Anthrenus verbasci* shows a circannual pupation rhythm and phase shifts are induced by photoperiod changes (Nisimura and Numata 2001). In the present study, larvae kept under a constant short-day photoperiod were exposed to a long-day photoperiod for 4 weeks at 20°C. Although an exposure to a