## UPTAKE OF GLUTAMINE AND ULTRASTRACTUAL CHANGES IN LARVAL CELLS DURING SEA URCHIN METMORPHOSIS

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Metamorphosis of the sea urchin *Hemicentrotus pulcherrimus* can be induced by L-glutamine (Gln) or a natural cue, green algae (*Ulvella* sp.) In the metamorphosis induced by Gln, the larvae began to retract their arms at first, and then everted their echinus rudiments (ER). Meanwhile in larvae treated with *Ulvella*, the ER everted before the retraction of larval arms. Sea urchin larvae have two types of epithelia, the squamous epithelium and the columnar epithelium. According to the electron microscopy, we first found some cellular changes in larvae at 6 hr-treatment with Gln. Larval arms seemed to keep length until 6 hr after the start of Gln-treatment. After 24 hr-Gln treatment, the nuclear chromatin markedly condensed in the squamous epithelial nuclei of larval arms and body including the esophagus. Most nuclei in which chromatin had condensed were shown positive to TUNEL assay which detected DNA fragmentations. In columnar epithelia of these larvae, the nuclei chromatin condensation was observed in less cells. These results suggest that the two types of epithelia react differently to the Gln treatment. In addition, we preliminarily examined a time course experiment of $^{14}$C-labeled Gln. Radio activity in a larva continuously increased during the 24 hr Gln-treatments, and most of the activity were detected in methanol soluble phase. It has been found that Gln incorporated in larvae by Gln-treatment is metabolized to glutamic acid and other amino acids (Yazaki and Nishigori unpublished data). To make clear the difference in reactivity to the Gln-treatment between two epithelia and to identify the causal substance to give size to larval cell death, we will analyze the metabolism of Gln, especially the location of Gln-incorporation and the timing of its metabolism.

## Immunohistochemical localization of $T_3$-responsive tissues in the adult transgenic frogs

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This study examined the responsiveness of thyroid hormone responsive element (TRE)-containing promoter sequence to thyroid hormone (TH) utilizing *Xenopus laevis* carrying a transgene containing 5'-upstream region of TRbetaA1 gene and sequence of green fluorescent protein (EGFP) gene. We showed that the transgenic adult frogs were responsive to exogenous TH, a high responsiveness being seen in brain, small intestine, kidney, and bones. Thus, TH not only regulates the metamorphosis, but also might play some physiological role(s) in these adult tissues.

## EXPRESSION OF MRNAS ENCODING WATER CHANNEL PROTEINS IN THE VENTRAL SKIN OF THE JAPANESE TREE FROG, *Hyla areborea japonica*

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Water channel proteins called aquaporins (AQPs) play a key role in water movement across the cell membrane. Since adult anuran amphibians absorb water through the specialized regions of ventral skin, water channels were considered to exist on these regions. In the present study, we have identified two cDNAs encoding AQPs from the ventral skin of Japanese tree frogs by RT-PCR amplification. Sequence analysis revealed that one AQP consisted of 271 amino acid residues with 86%, ca. 56%, and ca. 28% identity to toad AQP-t3, mammalian AQP-5, and mammalian AQP-3, respectively. This tree frog AQP was further predicted to comprise six putative bilayer-spanning segments and five connecting loops (A–E) with the Asn-Pro-Ala (NPA) motif in the loops B and E, which are characteristic of the members of AQP family. The 2nd NPA motif was flanked by a cysteine corresponding to the mercurial-inhibitory site common to all the AQPs except AQP-4. Two N-glycosylation consensus sites were present in the loop C. The other tree frog AQP was similar to vasopressin-regulated water channels with ca. 94% identity to toad AQP-t2 and ca. 62% identity to mammalian AQP-2. The results suggest that these tree frog AQPs may be involved in the water absorption through the ventral skin.

## SYNCHRONIZATION OF INTRACELLULAR CALCIUM OSCILLATIONS IN PREOPTIC NEUROSECRETORY NEURONS OF RAINBOW TROUT

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We examined spontaneous changes of intracellular calcium concentrations ($[Ca^{2+}]_i$) in preoptic neurosecretory neurons of rainbow trout to characterize their basal neuronal activity. The brain of immature rainbow trout was cut at the mid-sagittal plane, and preoptic neurons just beneath the third ventricle were exposed by a treatment with 0.1% collagenase and 0.1% trypsin solution. They were then labeled with a calcium indicator dye, Oregon Green 488 BAPTA-1, and the fluorescent images were recorded every 5 sec for 30–60 min by a confocal laser scanning microscope. The recorded cells were identified by double color immunofluorescence for vasotocin and isotocin. Both vasotocin-positive and isotocin-positive cells showed $[Ca^{2+}]_i$oscillations which were synchronized within immunohistochemically identical cell groups. Non-neurosecretory cells showed asynchronous $[Ca^{2+}]_i$ oscillations. In 21 fish, vasotocin cells showed oscillations at an interpulse interval of 4.5 +- 0.3 min and a duration of 39.9 +- 1.5 sec, whereas isotocin cells showed that of 7.5 +- 0.7 min and 119.6 +- 10.9 sec. Depolarization with increasing extracellular $[K^+]_i$ shortened the interpulse interval. The synchronization of neuronal activity in neurosecretory neurons may be important for the release of hormones.

## BOMBYXIN, AN INSULIN FAMILY PEPTIDE OF INSECTS, ACTIVATES ENERGY METABOLISM IN THE SILKWORM *Bombyx mori*

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It has been shown that bombyxin enhances the consumption of the major storage carbohydrates of *Bombyx mori* larvae such as hemolymph trehalose and fat body glycogen. However, how the degradation products of these carbohydrates are used is not known. In the present study, we investigated the changes in the quantity of lipids and in the rate of oxygen consumption of the animals after injection of bombyxin into neck-ligated fourth instar larvae of *B. mori*, for the purpose of revealing the metabolic pathway which is regulated by bombyxin. The decrease in hemolymph trehalose in the first 3 hr after injection was about 40% greater than that in saline-injected control. However, no effect of bombyxin was found on both hemolymph lipid concentration and lipid content in the fat body. By contrast, respiration rate of the animals was significantly increased by bombyxin injection: oxygen consumption in the first 3 hr in bombyxin-injected animals was about 20% larger than that in control animals. These results suggest that bombyxin does not stimulate biosynthesis of lipids from carbohydrate store, but activates energy metabolism.

## PHYSIOLOGICAL ROLE OF PLACENTAL LEUCINE AMINOPEPTIDASE / OXYTOCINASE IN THE KIDNEY

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Placental leucine aminopeptidase (P-LAP) / oxytocinase (EC 3.4.11.3), which is secreted from placenta to the maternal serum during gestation, is believed to play an important role in the maintenance of pregnancy via the inactivation of oxytocin. The expression of P-LAP, however, was observed in various tissues such as kidney, brain and heart, suggesting that this enzyme also has some other functions.

In order to clarify the physiological role of P-LAP in the kidney, we prepared the recombinant human P-LAP expressed in Chinese hamster ovary cells and examined its hydrolytic activity toward kidney-related peptide hormones by means of high-pressure liquid chromatography analysis. Among the peptides examined, P-LAP hydrolyzed vasopressin as well as angiotensin III and converted kallidin, a decapeptide generated by the action of tissue kallikrein highly expressed in the kidney, to bradykinin.

Immunohistochemical analysis revealed that P-LAP was localized to the distal tubule and the collecting duct cells both in the human and rat kidney. This result implies the co-localization of P-LAP with vasopressin receptors and tissue kallikrein, since the former is known to be distributed both in the distal tubules and collecting ducts and the latter in the distal tubules.

These results, together with the analysis of the subcellular localization and its changes using kidney-derived cell lines, suggest that renal P-LAP is involved in the control of water-electrolyte balance and blood pressure by regulating the levels of vasoactive peptides such as vasopressin and kinins.