CHANGES OF WING COLOR PATTERN BY MOLSIN INJECTION INTO THE PUPA OF *PAPILIO XUTHUS*

Yoshishige Umebachi, Minoru Osanai
Formerly, Department of Biology, Faculty of Science, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan

Injection of Molsin (an acid carboxypeptidase of *Aspergillus saitoi*) into the pupa of *Papilio xuthus* (a swallowtail butterfly) causes perturbation of wing color pattern. Several kinds of yellow and black color pattern are obtained depending on developmental stage of the pupa injected. When the injection was performed in the 0-day-old pupa, the yellow markings in the peripheral area of hind-wings enlarged. The injection to the 1-day-old pupa caused the enlargement of yellow regions of forewings. This tendency was stronger in the underside than in the upperside. When the injection was performed in the 2-day-old pupa, the wings of yellow types decreased and melanized area enlarged.

GBP BLOCKING PEPTIDE EXIST IN OENOCYTOIDS

Yasuko Matsumoto, Yasunori Oda, Masahide Uryu, Yoichi Hayakawa
Biochemistry Laboratory, Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

Growth blocking peptide (GBP) is an insect cytokine with multiple biological activities. A GBP binding protein was isolated from the hemolymph of the armyworm suppresses the GBP-induced activation of plasmatocytes *in vitro*. Northern and western blot analyses indicated that the binding protein is synthesized in hemocytes. Immunoelectron microscopic analysis confirmed that indirect immunoreactive signals were mostly localized in a morphotype of hemocytes, oenocytoids. We recently found that GBP induces a hemolysis of oenocytoids by which the binding protein is released. Further, the GBP-induced oenocytoids lysis took place approximately 20min after plasmatocytes began to spread by GBP, thus suggesting that the GBP-induced activation of plasmatocytes was followed by the release of the binding protein through the oenocytoids lysis. This observation supported the hypothesis that the binding protein released from the oenocytoids is to serve as a scavenger of GBP.

CONTROL OF GENE EXPRESSION OF TETRAHYDROBIOPTERIN BIOSYNTHETIC ENZYMES IN HSG CELLS BY ANDROGEN

Akemi Inaba[1], Sumiko Negishi[2], Kengo Fujimoto[1], Akishi Fukatami[3], Setsuko Katoh[1],
Departments of [1]Biochemistry and [3]Biology, Meikai University School of Dentistry, Sakado, Saitama 350-0248, Japan and [2]Department of Biology, Keio University, Yokohama, Kanagawa 223-8521

Nitric oxide (NO) regulates protein secretion mediated by a cGMP-Ca^{2+} signaling pathway in salivary gland. We previously demonstrated the gene expression of neuronal NO synthase (NOS) and biosynthetic enzymes of tetrahydrobiopterin (BH4), an indispensable cofactor of NOS in rat salivary gland by *in situ* hybridization. In acinar and duct cells of submandibular and parotid glands, we detected all of enzymes involved in the biosynthetic pathway of BH4. Moreover, localization of hybridization signals for nNOS was similar to that for BH4 biosynthetic enzymes. Multipotential human submandibular gland (HSG) cell line by androgen is induced to duct-like cells, which produces NGF and EGF. Previous study suggested enhancement of BH4 production by NGF. In this study, we examined the gene expression of biosynthetic enzymes of BH4 in HSG cells by androgen. In total RNA extracted from HSG cells treated with androgen, we analyzed the change of the gene expression of BH4 biosynthetic enzymes using real time quantitative PCR by time-dependent manner.

ANALYSIS OF THE ACTIVATION MECHANISM OF 20S PROTEASOME INDUCED BY ULTRASONIC WAVE

Toru Hayashi[1], Masahiko Yano[2], Masumi Yasutomi[3], Katsutoshi Ishikawa[4], Shinpei Yamada[4]
[1]Graduate School of Science and Technology, Shizuoka University, Shizuoka, Shizuoka 422-8529, Japan, [2]Miyakoda research institute, Suzuki Co. Ltd., Hamamatu, Shizuoka 431-2103, Japan, [3]Lab, of Biology, Aichigakuin university, Nisshin, Aichi 470-0195, Japan and [4]Department of Biology and Geosciences, Faculty of Science, Shizuoka University, Shizuoka, Shizuoka 422-8529, Japan

We reported that the classical three peptidase acitivities, chimotrypsin-like, trypsin-like and peptidylglutamyl-peptide hydrolyzing activity of *Xenopus* ovary 20 S proteasome was activated up to 100 times by the ultrasonic wave irradiation of 60 kHz at last meeting of Japan zoology society. To investigate this mechanism, we examined a lot of factors that affect the activation induced by the ultrasonic wave irradiation. The result suggests that hydrogen peroxide and free radical that are generated by the ultrasonic wave irradiation are not the direct cause of activation, and that the mechanism of the activation seems to be basically same as that by proteasome activator such as SDS. Furthermore, the activation by the ultrasonic wave irradiation was suppressed significantly in the pressence of trypsin-like substrate, suggests that comformational change of 20 S proteasome is induced by addition of trypsin-like substrate.

CLONING OF cDNA ENCODING CP-19K, UNIDENTIFIED CEMENT PROTEIN IN BARNACLE UNDERWATER ADHESIVE PROTEINACEOUS COMPLEX

Kei Kamino
Marine Biotechnology Institute

Barnacle is marine sessile organism, and typical fouling animal. After metamorphosis of the larvae, they start to secrete underwater adhesive protein-complex called cement to firmly settle onto substrate. Recent our development of the method to render the insoluble barnacle cement-complex soluble allow to identify four cement protein components, although cement protein (cp)-19 k were remained to be characterized due to problem in the purification. In this study, cp-19 k was purified by 2D-PAGE, and the N-terminal sequence was determined. The sequence information was applied to identify the cDNA from barnacle cDNA library.

THE ROLE OF LYSOZYMES IN NITRONGEN METABOLISM OF TERMITES

Ai Fujita, Isamu Shimizu, Takuya Abe
Center for Ecological Research, Kyoto University, Kamitanakami Otsu, Shiga 520-2113, Japan

Three kinds of PCR-product cDNA that encode premature lysozyme peptides (Rs-Lys1, Rs-Lys2 and Rs-Lys3) were cloned from workers of Japanese damp-wood termite, *Reticulitermes speratus*. Aliment of these sequences with those of other insect lysozymes showed that the cDNAs encode lysozyme homologues with putative signal peptides, insertions einsertions eight amino acids long, and a relatively long C-terminus (13–17 amino acids). A neighbour joining tree constructed using the amino acid sequences indicated that the termite lysozymes are related to those of mosquitoes and lepidopterans. Southern-blotting analysis identified single copies of these lysozyme genes in the termite. RT-PCR and *in situ* hybridization experiments showed the expression site of these lysozymes genes in the worker termites. Here, we discuss the possible digestive function of these lysozymes.

HAS THE COTESIA KARIYAI POLYDNAVIRUS EVOLVED FROM A NUCLEOPOLYHEDROVIRUS?

Kohjiro Tanaka, Seiji Tsuzuki, Yoichi Hyakawa
Institute of Low temperature Scince, Hokkaido University, Sapporo 060-0819, Japan

Polydnaviruses are obligate symbionts with certain parasitic wasps. The importance of the surface of Cotesia kariyai polydnavirus (CkPDV) has been indicated by our finding the presence of the surface protein (termed IEP for immunoevasive protein) mediating immunoevasion by the wasp from the encapsulation reaction of the host hemocytes. A major envelope protein with a molecular mass of approximately 60 kDa (EP60) has been isolated and characterized for comparison with IEP. The EP60 synthesis was detected 36 h earlier than the IEP synthesis in the specific cells of the female ovaries called the calyx cells where CkPDV replicates, while IEP is synthesized in the oviduct cells in which CkPDV paticles are stored; thus, the CkPDV particles likely have two exterior layers, the envelope and IEP layers. Electron microscopic observation demonstrated this speculation: the envelope layer is covered with the IEP-rich fragile layer that should contribute to evade the host defense reaction. Furthermore, we demonstrated that the CkPDV genome encodes the EP60 gene whose primary structure shows a significant homology with that of the nucleopolyhedrovirus P35 gene.

DIFFERENTIAL EXPRESSION OF TWO CATHEPSIN Es IN *XENOPUS LAEVIS* FOREGUT DURING THE METAMORPHOSIS

Masayuki Ikuzawa[1], Shigeki Yasumasu[1], Ken-ichiro Kobayasi[1], Kohji Nomura[2], Ichiro Iuchi[1]
[1]Life Science Institute, Sophia university, Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan and [2]Proteome Group, Tokyo Metropolitan Inst. of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan

Cathepsin E (CE) was purified from foregut of tadpole of *Xenopus laevis* as a mature dimeric form. It was a typical CE molecule among aspartic proteinases in pH dependence of proteolytic activity, susceptibility to pepstatin, and having N-linked high-mannose type oligosaccharide chains. We isolated two cDNAs for CE (CE1 and CE2) from adult stomach. Partial amino acid sequence of N-terminal region of the purified CE coincided with the sequence predicted from the CE1. Northern blot analysis and in situ hybridization clarified that CE1 mRNA was highly expressed in surface mucous cells and gland cells constituting larval type epithelium of the pre-metamorphic tadpole foregut. After the metamorphosis began, the CE1 mRNA drastically decreased in amount, and subsequently, both CE1 and CE2 mRNAs gradually increased. The decrease correlated with degeneration of the larval epithelium, while the increase did with formation of adult type epithelium. It is probable that cathepsin E gene expression is differentially regulated in epithelial cells during the metamorphosis-associated foregut-to-stomach remodeling.