

defection in head structure.

#### IDENTIFICATION AND CLONING OF GROWTH-BLOCKING PEPTIDE (GBP) RECEPTOR

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GBP is an insect cytokine isolated from *Pseudaletia separata* that possesses diverse biological activities such as larval growth regulation, cell proliferation, and activation of immune hemocytes. For the purpose of characterizing GBP receptor (GBPR), we attempted to purify the GBPR from *P. separata* hemocytes. Hemocyte membrane was solubilized by detergent, applied to GBP-immobilized column and eluted by acidic buffer. As judged by SDS-PAGE, 4 proteins with molecular masses of 106, 103, 95 and 88 kDa respectively, were detected as proteins with affinity for GBP. According to partial peptide sequences, we cloned two cDNAs encoding p88 and p95, respectively. The primary structures of p88 and p95 are highly homologous each other and consist of signal peptide, 2 or 3 low-density lipoprotein receptor (LDLR) domains and subsequent 5 or 7 EGF-like domains, respectively. Although the primary structures of both proteins display limited homology to the extracellular portion of LDLR family, they lacked both transmembrane and intracellular domains. Based on these observations, we hypothesized that p88 and p95 act as a ligand binding subunit for GBPR with unidentified membrane-spanning subunit.

#### PURIFICATION AND CHARACTERIZATION OF CASEIN KINASE 2 (CK2) FROM THE EGGS OF THE SILKWORM, *BOMBYX MORI*

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Casein Kinase 2 (CK2) has been purified from the silkworm eggs by several column chromatographic procedures. The purified enzyme required ATP and GTP as phosphate acceptor and phosphate donors, and the activity was inhibited by heparin. Furthermore, poly-Lys and poly-Arg stimulated the activity. These characters coincided with the properties of the various animals CK2s. In order to clarify the physiological roles of CK2 during the early development, we tried to compare between the non-diapause eggs and the diapause eggs until 60 hours after the oviposition. The activity of CK2 during the development with the non-diapause eggs, the phosphorylated signals were recognized through the all stages, and the signals were getting stronger until at the end of the stages. On the other hand, in the diapause eggs, the strongest phosphorylated signals were recognized around at 24 hours after the oviposition, and the signals were getting weaker until at the end of the stages. These results suggest that the phosphorylation by CK2 in the silkworm eggs have an important role for development both non-diapause eggs and diapause eggs.

#### EFFECT OF TESTOSTERONE IN TETRAHYDROBIOPTERIN BIOSYNTHESIS OF THE HUMAN SALIVARY GLAND (HSG) CELLS

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An intracellular concentration of calcium plays an important role in the regulation of protein secretion from salivary glands. It is suggested that the cGMP/nitric oxide (NO) signaling pathway is involved in this process. NO is synthesized from L-arginine by NO synthase (NOS). For the activation of NOS, tetrahydrobiopterin (BH4) is known to be an indispensable cofactor. We previously showed that mRNAs of NOS and BH4 biosynthesizing enzymes [GTP cyclohydrolase I (GTPCH), pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SPR)] were involved in rat salivary (submandibular and parotid) glands, and human salivary gland (HSG) cells using analyses of *in situ* hybridization and RT-PCR, respectively. Moreover, we demonstrated that the expression of GTPCH (the rate-limiting enzyme of BH4 biosynthetic pathway) gene in HSG cells was enhanced by culture with androgen in a time-dependent manner by real-time quantitative PCR analysis. In this study, we oxidized extract of HSG cells cultured with androgen by iodine and examined contents of BH4 and change of BH4 biosynthesis in HSG cells using HPLC analysis.

#### EXPRESSION OF PROPEPTIDE-LIKE CYSTEINE PROTEINASE INHIBITORS

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Mouse activated T-lymphocytes express cytotoxic T-lymphocyte antigen (CTLA-2), which is homologous to the proregion of mouse cathepsin L. We have also identified a similar protein, *Bombyx* cysteine proteinase inhibitor (BCPI), in the silkworm *Bombyx mori*. BCPI is a strong and highly selective inhibitor towards cathepsin L-like cysteine proteinases just like the propeptides. We found a similar propeptide-like protein in *Drosophila* (D/CTLA-2). Expressed D/CTLA-2 in *E. coli* exhibited selective inhibition towards cathepsin L-like cysteine proteinases. D/CTLA-2 mRNA was predominantly detected in yolk cells, garland cells, prothoracic gland of the early embryo by *in situ* hybridization. Studies of the expression in larvae are in progress. Recently, we found that expressed CTLA-2alpha was also a selective inhibitor towards cathepsin L-like cysteine proteinases. Strong expression of the CTLA-2alpha was detected in placenta of mouse. It is known that several lysosomal cysteine proteases related to cathepsin L are specifically expressed in placenta. All these results suggest unique biological functions of these novel inhibitors.

#### A NEW PROTEASE OF THE PROPHENOLOXIDASE (proPO) CASCADE IN THE LARVAL HEMOLYMPH OF THE SILKWORM, *BOMBYX MORI*

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In insects, the proPO cascade is triggered by minute amounts of microbial cell wall components, followed by the sequential activation of protease zymogens and proPO. Of the components of the proPO cascade, the recognition proteins with special affinity to  $\beta$ -1,3-glucan ( $\beta$ G) or peptidoglycan (PG), proPPAE (zymogen of proPO-activating enzyme), proPO and proBAEEase (which was found as a zymogen of an enzyme hydrolyzing N<sup>ε</sup>-benzoyl-L-arginine ethyl ester) have been purified. ProBAEEase lies on the pathway diverging from the one for the activation of proPO. We recently purified a new serine protease zymogen (referred to as proS) of the silkworm proPO cascade, and demonstrated that proS is essential for the activation of proPO and proBAEEase. In this study, we purified the active form of proS (referred to as S). When S was added to silkworm plasma in the absence of  $\beta$ G or PG, both activities of BAEEase and PO appeared. The incubation of proBAEEase with S caused the conversion of the zymogen to the lower molecular form with its enzyme activity. However, the S did not appear to activate directly proPPAE, implying the involvement of another factor(s) for the activation of proPPAE by S.

#### STUDIES ON THE INSECTICIDAL MECHANISM OF A METALLOPROTEASE-LIKE INSECTICIDE

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Most of the armyworm larvae *Pseudaletia separata* are killed within 2-3 days by parasitization with the wasp *Cotesia kariyai* when they are simultaneously infected by the entomopathogen *Serratia marcescens*. Previous study elucidated that this insecticidal effect was mainly due to metalloprotease-like insecticide (MPLI) secreted from *S. marcescens*. The present study was conducted to elucidate the cause of the death of larvae injected with MPLI. Injection of MPLI caused elevation of hemolymphal dopamine concentration followed by the elevated levels of brain dopamine in the larvae. Radiolabelled dopamine injected into the hemocoel was detected in the brain of larvae injected with MPLI. Transmission electron microscopy showed decrease in thickness and density of brain sheath layer in larvae injected with MPLI. Further, electron microscopic and TUNEL staining analyses showed a significant increase of apoptotic cells in the brain after MPLI-injection. Based on these results, we concluded that MPLI-injected larvae might have suffered mortal damage through increased apoptotic cell death in the brains caused by an influx of dopamine from hemolymph.

#### PURIFICATION AND CHARACTERIZATION OF CHYMOTRYPSIN-LIKE PROTEASES IN THE INTESTINE OF SEA URCHIN, *H. PULCHERRIMUS*

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The alimentary canal of sea urchin is morphologically composed of pharynx, esophagus, foregut and hindgut with no secretory organs and there are few reports dealing with proteases there. We found that chymotrypsin-like activity was rather strong in the gut crude extract of *H. pulcherrimus*, as compared to the trypsin-like and elastase-like activities, and that it was high in the foregut, where secretory cells were abundantly distributed. We purified these chymotrypsin-like proteases. Chymotrypsin-like activity was separated into four peaks at hydrophobic chromatography step, and was designated C-I to C-IV according to the order of elution, respectively. C-I and C-II were further purified. They were similar to ordinary chymotrypsins with respect to the optimal pH of 8.0, the sensitivity to chymostatin and PMSF, but had unique properties. Namely, the molecular weight of 35kDa estimated by SDS-PAGE, was considerably high. Furthermore, the N-terminal amino acid sequences of these were almost identical to each other, but showed no similarity to those of any other chymotrypsins.