DDC protein in epidermal cells under the blackish stripes was observed immediately after the ecdysis. Furthermore, the injection of DDC inhibitor, 3-hydroxybenzylhydrazine dihydrochloride, before the last larval ecdysis, blocked the DDC activity and deprived the blackish color on the epidermis and localization of DDC. Therefore it is proposed that, during the ecdysis, the rapid cell proliferation occurs under the blackish stripes, where melanization is induced by the enhancement of DDC activity.

EEFFECT OF GBP BINDING PROTEIN TO DEVELOPMENT IN *PSEUDALETIA SEPARATA*

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Growth blocking peptide (GBP) is an insect cytokine with multiple biological activities. GBP binding protein (GBP-BP) suppresses the GBP-induced activation of plasmatocytes *in vitro*. GBP-BP is synthesized in oenocytoids, a class of hemocytes, and is thought to be released into hemocoel through the GBP-induced lysis of oenocytoids *in vivo*. Recently, we found that GBP-BP concentration in hemolymph increased after the last larval ecdysis, during which GBP concentration conversely decreased. In order to investigate the effect of GBP-BP on larval development, anti GBP-BP antibody was injected into last instar larvae. Injection of the anti GBP-BP antibody caused a significant delay in pupation, suggesting that the anti GBP-BP antibody neutralized the GBP-BP effect on larval development. Because one of the most important functions of GBP is the regulation of larval growth, it is reasonable to expect that GBP-BP could control the larval growth rate though binding with GBP.

A NOVEL CYTOKINE ISOLATED FROM THE HEMOLYMPF OF THE BLOWFLY *LUCILIA CUPRINA*

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The ENF peptides including Growth-blocking peptide (GBP), paralytic peptide (PP) and plasmatocyte-spreading peptide (PSP) are only cytokines which have been found in insects. These peptides exerts a variety of biological functions: larval growth regulation, paralysis induction, cell proliferation and hemocyte stimulation. These peptides have been identified only in Lepidopteran insects. In the present study, we isolated a potent cytokine that activates the blowfly hemocytes from the hemolymph of the last instar larvae of *Lucilia cuprina*. It also stimulates the proliferation of insect High five cells. The sequence of the cytokine was determined: H-Thr-Ile-Leu-Ser-Ala-Pro-Ser-Asn-Cys-Glu-Glu-Thr-Asp-Phe-Lys-Gly-Arg-Cys-Leu. A cDNA coding for this cytokine was cloned by a combination and PCR and cDNA library screening. The cloned cDNA was 555 base pairs in length, and an open reading frame encodes a pre-pro-peptide of 112 amino acid residues in which the cytokine molecule is localized at the C-terminal region.

CHARACTERIZATION OF UBIQUITIN-CONJUGATING ENZYME SPECIES RESPONDING TO CELL STRESSES

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Ubiquitin system is involved in degradation of abnormal proteins that result from cell stresses. Ubiquitin-conjugating enzymes (E2) catalyze, either directly or indirectly, covalent ligation of ubiquitin to target proteins in cooperative with ubiquitin ligases (E3). In all eukaryotes, substrate selectively of the reactions is mediated in part by members of a large family of E2, which consists of more than ten species. However, little is known about the functional responsibilities of each E2 species. Recently, we have developed the immunoaffinity technique with anti-ubiquitin antibody, which is applicable to isolation of ubiquitin-protein conjugates from biological materials. Using this method, several distinct species of E2-ubiquitin thioester intermediates, of which levels were increased in response to stresses (e.g. heat shock and ischemia), were identified from human leukemia cells and murine brain tissues. In addition, we have now succeeded in quantifying free and ubiquitin-thioester forms of these E2 in other cells by using a combination of immunoprecipitation and western blot. From these findings, features of E2 species responding to cell stresses will be discussed.

DESIGNING OF UNIVERSAL PCR PRIMERS TO AMPLIFY BARNACLE UNDERWATER ADHESIVE PROTEIN HOMOLOGOUS GENES

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Barnacle is sessile crustacean and the proteinaceous underwater adhesive is called cement. Six cement proteins were so far identified as cement proteins, which are designated to *Megabalanus rosa* cement protein (Mrcp)-100k, 68k, 52k, 20k, 19k, and 16k. Two cDNAs corresponding to cp-100k and cp-68k were also isolated from *Balanus amphitrite*. In this study, universal PCR primers were designed to amplify barnacle underwater adhesive protein homologous genes. Three cement protein genes were successfully amplified from *Balanus albicostatus* by using the universal primers. Sequence diversity and amino acids bias would be discussed in this presentation. This work was performed as a part of The Industrial Science and Technology Project, Technological Development for Biomaterials Design based on Self-organizing Proteins, supported by New Energy and Industrial Technology Development Organization (NEDO).

LOCALIZATION OF PHOTORECEPTORS, GnRHs, AND GnRH RECETORS IN THE NEURAL COMPLEX OF THE ASCIDIAN *CIONA INTESTINALIS*

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GnRH is a key regulator of the hypothalamo-pituitary-gonadal axis in vertebrates, but its roles and regulations are little known in ascidians. The ascidian spawn in response to light following darkness. Injection of synthetic GnRH into various sites can also elicit the spawning in ascidians. We hypothesized that light affects the spawning via the GnRH systems in ascidians. In a previous study, we showed that light inhibits spontaneous discharges of the GnRH immunoreactive cells in the cerebral ganglion of the ascidian *Halocynthia roretzi*. To elucidate the relationship between photoreception and the GnRH systems in ascidians, we have isolated and characterized cDNA clones encoding proteins involved in photoreception and phototransduction, GnRHs, and GnRH receptors in the ascidian *Ciona intestinalis*. We analyzed mRNA and protein localization of these factors in the neural complex by *in situ* hybridization and immunocytochemistry.

EFFECTS OF INSULIN-LIKE GROWTH FACTOR I AND SALMON GnRH ON GTH SUBUNIT GENE EXPRESSION IN MASU SALMON PITUITARY CELLS *IN VITRO*

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Effects of Insulin-Like Growth Factor I (IGF-I) and salmon GnRH (sGnRH) on expression of GTH subunit (α, FSHβ and LHβ) genes were examined in masu salmon (*Oncorhynchus masou*) using primary pituitary cultures. Pituitaries were taken from fish in April (early maturation), in June (maturing) and in September (spawning). The levels of GTH subunit mRNAs in the pituitary cells were measured by real-time PCR. In the males, three subunit mRNA levels were increased by sGnRH in April, but these effects were abolished in combination with IGF-I, whereas in September sGnRH and IGF-I synergistically increased three subunit mRNA levels. In the females, sGnRH tended to decrease three subunit mRNA levels in April and co-administration with IGF-I antagonized these effects. In September, sGnRH increased the levels regardless of IGF-I. These results indicate that IGF-I differentially modulates effects of sGnRH on GTH subunit gene expression, depending on reproductive stage and gender.

THREE GnRHs AND FIVE GnRH RECEPTORS IN THE SPOTTED GREEN PUFFERFISH *TETRAODON NIGROVIRIDIS* AND A NOVEL CLASSIFICATION OF VERTEBRATE GnRH RECEPTORS

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GnRH (gonadotropin-releasing hormone) plays a pivotal role in the regulation of reproductive functions. It has become a general notion that multiple GnRH isoforms and multiple types of GnRH receptor (GnRHR) are distributed in a wide range of tissues within single organisms. We have identified three GnRH and five GnRHR isoforms in the spotted green pufferfish *Tetraodon nigroviridis*. The *Tetraodon* possesses the compact and smallest genome among vertebrates measured to date. Their mRNAs showed distinct but widespread expressions, though the roles of GnRH outside the hypothalamus-pituitary-gonadal axis remain largely unknown. Molecular phylogenetic analysis incorporating our new data from the present study revealed that vertebrate GnRHRs are roughly classified into four groups: types 1 to 4. The five GnRHRs cloned in the present study were divided into two distinct lineages: types 1 and 2. It was further confirmed that two distinct GnRHR lineages exist in single fish species.