transfection assays were conducted to examine the effects of AhR-Arnt on ecdysteroid signaling. Cotransfection of any possible combinations of AhR-Arnt isoforms attenuated transcriptional repression by unliganed EcR-USP. On the other hand, an ecdysteroid-enhanced reporter gene expression was differentially affected by different combinations of AhR-Arnt isoforms.

STUDY ON A CUTICULAR PROTEIN SYNTHESYZED AT INJURED AREA OF THE CUTICLE OF THE SILKWORM, *BOMBYX MORI*

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Insects' cuticle is an extracellular matrix composed of protein, carbohydrate and lipids, secreted from monolayer of epidermal cells. It covers the entire surface of the insect and serves as a protective barrier between internal tissues and environment. In addition, it has been recently shown that the injury of the cuticle causes several immune responses such as melanin synthesis or induction of the synthesis of anti-microbial peptide. This clearly shows the importance of the insects' cuticle for defense reactions against the pathogenesis. A cuticle of the silkworm, *Bombyx mori*, was injured with sandpaper. After 24 hr, melanization was observed at the wounded part of the cuticle. Then, cuticular protein was extracted with sodium acetate buffer and the resultant extract was subjected to the analysis by SDS-PAGE. When its electrophoresis pattern was compared with that from non-injured worm, a protein band specific to injured worm was observed. It seemed that this protein was synthesized in response to the injury. Currently we are trying to purify and characterize it.

MOLECULAR ANALYSIS OF A PROTEIN IN THE EGG OF THE HERMATYPIC CORAL, *FAVITES CHINENSIS*

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Very little is known about molecular mechanisms regulating vitellogenesis in corals. With the purpose of obtaining a molecular marker for vitellogenesis in a coral, we analyzed protein components of the egg in a reef-building coral, *Favites chinensis* and isolated a cDNA clone encoding a major soluble egg protein. In this study, two major soluble proteins with apparent molecular masses of about 76.5 kDa and 60 kDa were observed when proteins were extracted from the egg and separated using SDS-PAGE. The N-terminal amino acid sequence and an internal amino acid sequence of the 76.5 kDa protein were determined. We then isolated two overlapping cDNA fragments encoding the 76.5 kDa protein from the mesentery containing maturating oocytes by (1) RT-PCR using degenerate primers that were designed based on the amino acid sequences and (2) 3'RACE. Through sequence analysis of these fragments, a sequence of 525 amino acids was obtained. Among the proteins in sequence databases, vitellogenin of a white sturgeon exhibited the highest sequence similarity to the 76.5 kDa protein.

MOLECULAR ANALYSIS OF GALAXIN, A SOLUBLE SKELETAL MATRIX PROTEIN IN THE SCLERACTINIAN CORAL *GALAXEA FASCICULARIS*

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It has been suggested that macromolecules in the organic matrices may regulate calcification in the exoskeleton of corals. In order to clarify molecular mechanisms of calcification in corals, the organic matrix was extracted from the calcified exoskeleton of the scleractinian coral *Galaxea fascicularis* and a protein in the matrix was analyzed. One major protein with an apparent molecular mass of 53 kDa was detected in an SDS-PAGE analysis of the extract. PAS-staining indicated that the 53 kDa protein was glycosylated. A cDNA fragment encoding this protein was obtained. In this study, a cDNA containing the entire open reading frame for the 53 kDa protein was obtained. Analysis of the deduced protein sequence suggests that the protein, named galaxin, is synthesized as a precursor consisting of a signal peptide of 22 amino acids, a propeptide sequence of 23 amino acids, and a mature protein of 298 amino acids. Galaxin exhibits a novel amino acid sequence which is characterized by a tandem repeat structure with a unit sequence of about 30 amino acids, and by occurrence of two dicysteine repeats (Cys-Cys) at fixed positions in each of the repeat units.

CRUSTOCALCIN: A STUDY ON POTENTIAL FUNCTION OF A SKELETAL CA^{2+}-BINDING PROTEIN OF KURUMA PRAWN *PENAEUS JAPONICUS*

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Calcification is the deposition of inorganic calcium crystals in animal hard tissues and it is widely observed in many animal species. However, the molecular mechanism of this process is not studied in detail. In crustaceans, calcification of the exoskeleton takes place at a specific stage of the molt cycle (postmolt stage). Therefore, there is a possibility that some of the genes expressed only at this stage are involved in the calcification process. Using differential display technique and Northern blotting, four genes expressed specifically at this stage were isolated. Of these genes, a gene named DD4 encodes an acidic protein named crustocalcin of 831 amino acids, and contains a highly glutamate-rich region of 121 amino acids (E-rich region). The glutamate content in this region is approximately 48%. This protein has at least two Ca^{2+}-binding regions and one of them contains the E-rich region. An immunohistochemical study indicates that the protein is localized in some of the calcified regions of the exoskeleton. Potential function of this protein will be discussed.

ANALYSIS OF QUINONE TANNING IN INSECT CUTICLE

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Insect cuticle is an extracellular matrix composed of proteins, chitin and lipids, and functions as an exoskeleton. Its stiffness is important properties for the function of cuticle. It is thought that the cross-linking of cuticular components after the ecdysis brings the stiffness of the cuticle. This hardening process is called quinone tanning, which is catalyzed by laccase-type phenoloxidase (laccase). We purified activated laccase from silkworm pupal cuticles just after larval-pupal ecdysis. After digestion of the cuticle with trypsin, the extract was fractionated by a combination of column chromatography. Finally, laccase was eluted from Mono Q column as a single peak with FPLC. Molecular mass of the trypsin-activated laccase was estimated at 70 kDa by SDS-PAGE. We prepared an antibody to the laccase, and only one band with 110 kDa was detected in the epidermis by Western-blot analysis. It seems that this protein is precursor form of laccase. In the trypsin digested cuticlar extract, many bands were detectable by the antibody, and some of them were larger than 110 kDa. These data suggest that pro-laccase exists in cuticle as complexes covalently bound with another cuticle proteins.

X-RAY MICRODIFFRACTION OF SINGLE MYOFIBRILS OF INSECT FLIGHT MUSCLE REVEALS UNUSUALLY HIGH INTEGRITY OF MYOFILAMENT LATTICES

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The myofilaments of striated muscle are arranged in a hexagonal lattice within a sarcomere. In asynchronous flight muscle (AFM) of insect, it is known that the arrangement of the constituent protein molecules is so regular that a sarcomere can be regarded as a single protein crystal. By the use of 2 μm-sized X-ray microbeams, we were able to record diffraction patterns from single myofibrils of the flight muscle of a bumblebee, *Bombus* sp. Surprisingly, the patterns showed that the lattice planes of the hexagonal lattice were almost exactly in register over a distance of several millimeters, or ~1,000 sarcomeres in series. This implies that a whole myofibril of insect flight muscle can be regarded as a single protein crystal. To examine whether this was a structural feature common to all insects with AFM, we recorded diffraction patterns from myofibrils from various sources by using microbeams generated at the BL40XU and BL45XU beamlines of SPring-8. The result showed that the lattices of insects belonging to Hymenoptera and Diptera had high structural integrity, but those from Hemiptera (to which the most extensively studied *Lethocerus* belongs) did not.

DIGESTIVE β-GLUCOSIDASES FROM THE WOOD-EATING TERMITE, *NASUTITERMES TAKASAGOENSIS*

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β-Glucosidase activity was measured in the salivary glands and various gut regions of the wood-feeding termite, *Nasutitermes takasagoensis* (Shiraki). Strong activities were detected both in the salivary glands and the midgut. cDNAs corresponding to the activities were identified by reverse transcription-PCR (RT-PCR) with degenerate primers designed based on known β-glucosidase cDNA sequences in insects. The RT-PCR also confirmed the expression of the β-glucosidase in the salivary glands and the midgut but in none from the other part of the gut. The cDNA sequences obtained from the midgut were slightly different from those of the salivary glands. These results suggest that the expression sites of β-glucosidases have been altered in the course of evolution of termites as is the case of endo-β-1,4 -glucanases from termites, considering the fact that the termite that situates a phylogenetically basal lineage such as *Neotermes koshunensis*, produces β-glucosidase only in the salivary glands.