EXPRESSION ANALYSIS OF *N*-ACETYLTRANSFERASE (NAT) IN *BOMBYX MORI*

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Serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT) catalyzes *N*-acetylserotonin synthesis from serotonin. *N*-acetylserotonin is the direct precursor of melatonin (*N*-acetyl-5-methoxytryptamine). Although the functions of AANAT are well investigated in vertebrates, those in insects remain obscure. We analyzed the expression profiles of NAT mRNA in *Bombyx mori* to approach to the function of NAT both from circadian and developmental aspects. The NAT in pupal brains was intensely expressed during darkness and the expression pattern was photoperiod dependent. The significant changes of NAT expression in the midgut and fatbody during larval development were also observed. The results imply the important roles of NAT in insect physiology.

OCT-PROCTOLIN, A NOVEL PROCTOLIN-RELATED PEPTIDE, INDUCES CONTRACTION OF RADULA MUSCLE IN *OCTOPUS VULGARIS*

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We have identified a novel bioactive peptide, named oct-proctolin, from *Octopus vulgaris*. Oct-proctolin (PKYMDT) is structurally related to insect proctolin (RYLPT). The peptide was found to induce contraction of radula muscle. Immunohistochemical staining of the radula muscle by using the antiserun against oct-proctolin revealed that the peptide was localized in nerve fibers. The result indicates that the peptide is a neuropeptide and may act as a neurotransmitter or neuromodulator in the radula muscle of *Octopus*. To define the pharmacological characteristic of oct-proctolin, *Xenopus* oocytes that express *Drosophila* proctolin receptor was used. Proctolin activated the expressed receptor with an EC50 of 0.06 nM. On the other hand, the receptor had response to oct-proctolin with an EC50 of 1.2 microM, suggesting that there is probably a subtype of proctolin receptor or a novel receptor for oct-proctolin in *Octopus*.

IDENTIFICATION AND ANALYSIS OF TREHALASE FROM TARDIGRADES, *MILNESIUM TARDIGRADUM*

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Many species of tardigrades can tolerate the desiccation (anhydrobiosis). When they encounter the dried environment, they are dehydrated and enter the ametabolic state called 'tun'. They revive by rehydration and metabolism begins again. In these processes, trehalose is believed to play an important role to protect these animals from desiccation, although the gene network that regulates the anhydrobiosis is almost totally unknown. To elucidate the molecular basis of desiccation tolerance of tardigrades, we isolated a gene fragment encoding trehalase from terrestrial tardigrades, *Milnesium tardigradum* and will report its expression profile during anhydrobiosis.

SEARCH FOR MOLECULES THAT REGULATE COLD AND DESICCATION RESISTANCE IN *DROSOPHILA MELANOGASTER*

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Resistance to environmental stresses in insects is variable from one species to another. The mechanism of stress resistance is critical for insects to adapt to various environments. In *Drosophila*, basic molecular biological tools are available to study this mochanism. We investigated changes in signal transduction pathway under cold and desiccation stresses and sought the regulatory molecules of cold and desiccation resistance using mutants and gene overexpressed system. We ovserved that the mechanisms of cold and desiccation resistance are shared in some respects but found that some uniqueness in each process.

NEMATOCYST TOXIN OF CNIDARIANS KILLS ARTHROPODS

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Cnidarians feed on small crustaceans. When they are caught by fishing tentacles, they are killed immediately. This fact suggests some toxins which kill crustaceans specifically are included in the nematocyst. Crude nematocyst toxins of more than ten species of cnidarians were prepared by sonication. Not only crustaceans but also insects and spiders were killed immediately after injection. But Oryzias latipes was not killed. Moreover, small shrimps, Heptacarpus pandaloides,H.geniculata, H. minutus that inhabitthe Zostera zone with the hydrozoan, Gonionema vertans, Crangonaffinis and giant caprella were resistive to the toxin.The toxin is a protein, and themolecular weight is estimated nearly at 60,000 by gel filtration and ultrafiltration. The toxin is necessary for cnidarians to get food, and seems to be in common with cnidarians

GLUTATHIONE S-TRANSFERASE HAVING VANADIUM-BINDING ACTIVITY ISOLATED FROM A VANADIUM-ACCUMULATING ASCIDIAN, *ASCIDIA SYDNEIENSIS SAMEA*

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Several species of ascidians accumulate vanadium in their blood cells (vanadocytes) at high concentration and with high selectivity. Through the accumulation process, almost vanadium ions in the +5 oxidation state are reduced to the +3 oxidation state via the +4 oxidation state and stored in the vacuole of vanadocytes. For hunting new proteins involved in this phenomenon, we have tried to isolate vanadium-binding proteins from a vanadium-rich ascidian, *Ascidia sydneiensis samea*, by a vanadium(IV)-chelating column. In this study, we analyzed a vanadium-binding protein with a striking homology to glutathione S-transferase (GST) isolated and identified from the digestive systems previously, named as *As*GST. Using the recombinant *As*GST, we revealed that *As*GST bound not only with vanadium(IV) but also vanadium(V). We further demonstrated that *As*GST formed a dimmer like other GSTs and exhibited GST activity. Immunological analysis cleared that *As*GST was expressed in almost all of the major tissues. In particular the expression level in the digestive systems was exceptionally high.These results suggested that *As*GST might be involved in the process of vanadium accumulation in ascidians.

ANALYSIS OF NOVEL VANADIUM-ASSOCIATED PROTEIN (VANABINP) ISOLATED FROM BLOOD PLASMA OF *ASCIDIA SYDNEIENSIS SAMEA*

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Several species of ascidians accumulate vanadium selectively at high concentration in particular blood cells (so-called vanadocytes). Previously, we identified vanadium-associated protein from blood plasma of a vanadium-rich ascidian, Ascidia sydneiensis samea. Its amino acids sequence was similar to Vanabin1 and Vanabin2, which have been known as vanadium-binding proteins in cytoplasm of vanadocytes, in the point that this protein has eighteen cystein residues whose intervals were conserved among vanabins. Therefore, this protein was designated as VanabinP. In this study, we have identified gene expression cells and localization of VanabinP, and examined metal binding ability of it to vanadium(IV). The results indicated that VanabinP was transcribed from several blood cells, some cells in the connective tissue, and a part of branchial sac, although VanabinP protein was mainly localized in plasma. The metal binding assay revealed that VanabinP bound to 12-13 vanadium(IV) ions per a molecule with a dissociation constant of 2.8×10^{-3}M. These results suggest that VanabinP may play as the metallochaperones, which translate vanadium ions for some target tissues.

METAL ION SELECTIVITY OF A VANADIUM BINDING PROTEIN,VANABIN2, DERIVED FROM *ASCIDIA SYDNEIENSIS SAMEA*

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Ascidians selectively accumulate high levels of vanadium from seawater into their blood cells, called vanadocytes. Recently a family of vanadium binding proteins designated as Vanabins were isolated from a vanadium-rich ascidian *Ascidia sydneiensis samea*. The 3D structure of Vanabin2 among them revealed that a novel bow-shaped conformation with four α-helices connected by nine disulfide bonds. The ^{15}N HSQC perturbation experiments of Vanabin2 indicated that vanadyl cations, which are exclusively localized on the same face of the molecule, are coordinated by amine nitrogens derived from amino acid residues such as lysines, arginines, and histidines, as suggested by the EPR results. In the present study, to elucidate metal selectivity of Vanabin2, metal ion binding ability of recombinant Vanabin2 was analyzed by using a metal-affinity column to which one of several transition metals such as Cu^{2+}, VO^{2+}, and Zn^{2+} was chelated within the pH4.5 to 6.5 region. As a result, Zn^{2+} binding Vanabin2 increased in amount by changing pH from 4.5 to 6.5. While, amount of VO^{2+} binding Vanabin2 was unchanged. These results indicate that Vanabin2 can selectively bind to VO^{2+} in the acidic condition.