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Changes of Proteins during Epididymal Maturation in the Acid Extracts of Boar Spermatozoa: 2-D Gel Electrophoresis Analysis

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The changes in protein composition of the acid extracts of boar spermatozoa were analyzed by two dimensional (2-D) gel electrophoresis during epididymal transit of boar spermatozoa. Boar spermatozoa were collected from caput, corpus, and cauda epididymis and subjected to acid extraction at pH 4.0. Acid soluble fraction was then anlyzed by 2-D gel electrophoresis. Comparison of the gel patterns demonstrated thatthere are proteins whose quantities increased from caput to cauda, where as some proteins decreased in quantities from caput to caudal epididymis. When the intensity of each protein was compared quantitatively, D1-D6 (D1; 35.8 kd/ pI 4, D2; 35.8 kd/ pI 4.2, D3; 32.5 kd/ pI 3.95, D4; 32.5 kd/ pI 4.25, D5; 28.6 kd/ pI 3.8, D6; 28.6 kd/ pI 4.15) decresed significantly in their intensities from the caput, corpus to the cauda spermatozoa when C1 (41.9 kd/ pI 5.3, C2) and C2 (41.9 kd/ pI 5.6) were used as controls. On the other hand, I1-I3 (I1; 16.8 kd/ pI 5.4, I2; 19.1 kd/ pI 5.8, I3; 20.1 kd/ pI 6.4) proteins increased in their intensities from the caput to the cauda spermatozoa

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Purification and cDNA Cloning of a Low-abundance Protein from Larval Hemolymph of Greater Wax Moth, *Galleria mellonella*

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A low-abundance protein from larval hemolymph of *Galleria mellonella* was purified and partially characterized. The protein was temporally named Gm27 for its molecular mass on SDS-PAGE gel. Gm27 was present in both male and female, and is present during all developmental stages. From the protein, several fragmented peptides were prepared by in-gel digestion method, and sequenced using MALDI MS/MS. RT-PCR was conducted using degenerate primers designed from the internal amino acid sequences. 5'-RACE PCR was used to obtain the complete protein coding region sequence and 5'-UTR. The full length Gm27 cDNA sequence encodes a 234 amino acid polypeptide including a potential 15-amino acid signal peptide. The deduced amino acid sequence also contains potential *O*-linked glycosylation sites.

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Change of Iron-binding Proteins on Iron or *E. coli* Injection into a Cricket, *Gryllus bimaculatus*

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We studied the compositional change of iron-binding proteins (IBPs) in *Gryllus bimaculatus* haemolymph by *Escherichia coli* injection. The proteins were identified by gel filtration column chromatography (GFC, G3000sw, Tosoh Co.) and PAGE. IBP fractions from GFC were stained with Ferene S. Two types of haemolymph ferritin (hFt-1 and hFt-2) were observed from *G. bimaculatus* haemolymph. It was shown that the amount of hFt-1 was increased by FeCl3 injection, while hFt-2 was decreased. *E. coli* injection caused decrease of the amount of hFt-2, but did not affect to hFt-1. Meanwhile, another IBP was observed in a GFC fraction containing hFt-2. The protein was shown to have a molecular mass of about 80 kDa on a 10-20% SDS-PAGE gel. We supposed that the protein is *G. bimaculatus* transferrin, because the protein was increased by *E. coli* injection, and was cross-reacted against anti-horse transferrin.

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vSBP is Involved in Regulation of p27 Expression in Breast Cancer Cell Lines

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vSBP was identified as a binding protein of PLC1 SH domain and was also associated with p85a, PI3K regulatory subunit. vSBP interacted with PLC1 and p85 through PXXP motifs, and then decreased the growth rate. Expression of v SBP induced the increase of p27, CDK inhibitor. Although p27 inhibits the cell cycle, p27 is highly expressed in several cancer cells, especially breast cancer cells. Interestingly, v SBP was also expressed in breast cancer cell line MCF7 as well as p27. Moreover, vSBP has the same expressional pattern with p27 under condition of serum depletion in breast cancer cell line MDA MB 231. To investigate the relevance between vSBP and p27 in the point of expression, we introduced the vSBP sense or antisens (AS) into MCF7 and MDA MB 231 cells. In the case of MDA MB 231, vSBP and p27 were expressed under condition of serum depletion, and v SBP AS inhibited the increase of p27 at the same condition. However, growth rate of vSBP AS transfected MDA MB 231 was not inhibited by serum depletion. Furthermore, overexpression of vSBP mutant form 2mp that doesn't interact with PLC1 and p85 couldn't induce the increase of p27. As a result of transfection of vSBP sense and AS, p27 was increased or decreased by sense and AS respectively. The increase of p27 by vSBP sense induced the decrease of growth rate and the decrease of p27 by vSBP AS induced the increase of growth rate. However, vSBP AS has not same effect in soft agar culture. In the result of counting of colony, vSBP AS decreased the number of colony contrary to growth rate.