ANALYSIS OF REGULATORY SEQUENCES IN THE FIRST INTRON OF THE 30K PROTEIN GENE, THE PLASMA PROTEIN GENE OF THE SILKWORM, *BOMBYX MORI*

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The plasma proteins termed "30K proteins" are synthesized by the fat body cells of the silkworm, *Bombyx mori* in a stage-specific manner during larval development. To study molecular mechanisms by which expression of 30K protein gene is controlled, we have established the primary culture method for fat body cells of *B. mori*.

Chimeric genes containing the cis-regulatory sequences of 30K protein gene fused to the firefly luciferase structural gene were introduced into fat body cells using a method of electroporation. Optimum transient expression was obtained at 90 V and 1,070 μF capacitance using serum-free Grace's insect medium as an electroporation buffer. When the constructs were transfected into fat body cells, the luciferase gene was accurately transcribed under the control of the 30K protein gene promoter. We examined the effects of 30K protein intron 1 sequences on reporter gene expression. A transfected fusion gene containing the intron sequences was highly expressed in fat body cells relative to that lacking of the intron sequences. This result indicates that transcriptional enhancer element(s) located in 30K protein intron 1 are important for expression of 30K protein gene.

GENOMIC ORGANIZATION OF MOUSE PROLYL OLIGOPEPTIDASE

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Prolyl oligopeptidase (EC.3.4.21.26; POP, formerly known as prolyl endopeptidase) is widely distributed in mammalian tissues, catalyzing internal peptide bond hydrolysis of Pro-X. POP has been purified and characterized from many sources and its cDNA has been recently cloned from several species. Such studies suggest that POP is involved in a lot of biologically important events, but its physiological role remains to be elucidated. In this study, we isolated the genomic clones of POP from mouse genomic library and determined the genomic organization of mouse POP. We also sequenced the 5'-flanking region of this gene. This is the first information of POP gene structure and will be of great help to conduct future functional studies of POP.

ANALYSIS OF THE CHITIN BINDING DOMAINS IN LARVAL CUTICLE PROTEINS OF THE SILKWORM, *BOMBYX MORI*.

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Insect cuticle of is composed mainly of chitin and chitin-binding cuticle proteins. We have purified four larval cuticle proteins (LCPs) of the silkworm, *Bombyx mori*, and cloned cDNA sequences for these LCPs. Comparison of primary structures of the LCPs predicted from cDNA sequences showed that all four proteins share the conserved amino acid sequences.

To understand the function of the conserved domain of LCPs, a series of deletion mutants of LCPs was synthesized in *E. coli* as GST fusion proteins, and chitin-binding activity of each mutant was analyzed by chitin affinity chromatography. Mutant proteins containing the conserved domain showed chitin-binding activity, whereas mutant proteins lacking this domain failed to bind to the chitin column. The result suggests that the conserved domain is responsible for chitin-binding activities of LCPs.

MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL KALLIKREINS EXPRESSED IN MOUSE TESTIS.

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We found enzyme activity capable of activating single-chain tissue-type plasminogen activator (tPA) in conditioned media of mouse tumor cells (Lewis lung carcinoma and B16 melanoma cells). Further characterization studies of this enzyme suggest that it is a tissue kallikrein-like enzyme. To obtain a candidate cDNA clone for the enzyme, we carried out RT-PCR as primers synthesized on the basis of the conserved sequences of mouse kallikrein family using mRNA isolated from the tumor cells. In this way, we obtained a novel kallikrein cDNA fragment. Since the gene was found to be predominantly expressed in mouse testis, we screened the testis cDNA library. As a result, three novel kallikrein cDNAs were obtained. The expression of these genes increased during the development of testis and were localized in Leydig cells. These observations suggest that the novel kallikreins are involved in the testicular function of adult mice.

ANALYSIS OF cAMP SIGNALING PATHWAY IN THE FAT BODY OF THE SILKWORM, *BOMBYX MORI*.

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Major plasma proteins, '30K proteins', are synthesized in the fat body of the 5th instar larvae of the silkworm, *Bombyx mori*. Biosynthesis of 30K proteins is regulated in a stage- and tissue-specific manner at the level of gene transcription.

To elucidate regulatory mechanism of expression of 30K protein genes, concentrations of typical 2nd messengers in the fat body cells were studied during 5th larval instar. We detected a sharp peak of cAMP concentration in the fat body immediately after the 4th molt, at which transcription of the 30K protein genes is activated. Furthermore, we identified CRE (cAMP response element) consensus sequences in the 5' flanking region of the 30K protein genes. To understand the role of the cAMP signal cascade on the regulation of 30K protein genes, we attempted to isolate a cDNA clone homologous to the CREB (cAMP response element binding protein) that controls gene expression in response to the cAMP signal in many species. We cloned a 100 bp PCR fragment of the putative *Bombyx* CREB homologue. Cloning of a full-length cDNA clone of the *Bombyx* CREB is in progress.

THE CATALYTIC SITE OF CARBONYL REDUCTASE ACTIVITY OF SEPIAPTERIN REDUCTASE.

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Sepiapterin reductase (SPR) catalyzes the last step in the pathway of tetrahydrobiopterin (BH_4) biosynthesis from GTP. Furthermore, SPR shows activity of carbonyl reductase. SPR is important for animals to live, because SPR lacked mutant has not discovered yet. In this study, we cloned SPR cDNA and inserted into pTrxFus expression vector. Several mutants, $Ser^{158}\rightarrow Asp$, $Tyr^{171}\rightarrow Val$ and $Lys^{175}\rightarrow Ile$, were expressed and purified. The kinetic parameters of them for sepiapterin and carbonyl compound (1-phenyl-1,2-propanedione) were determined. These mutants showed similar Km values for sepiapterin and 1-phenyl-1,2-propanedione to those of wild-type SPR. However, in all mutants, k_{cat} against sepiapterin and 1-phenyl-1,2-propanedione were extremely lower than those of the wild-type SPR. These results suggested that a set of Ser^{158}, Tyr^{171} and Lys^{175} were indispensable for catalytic activity.