

Molecular Cloning of a cDNA Encoding Putative Apolipoprotein III from the Silkworm, *Bombyx mori*

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Objectives

Apolipoprotein III (apoLp-III) is a prototypical exchangeable apolipoprotein that is abundant in hemolymph of many insect species. Its function lies in the stabilization of low-density lipoprotein particles (LDLp) crossing the hemocoel in phases of high energy consumption to deliver lipids from the fat body to the flight muscle cells. But, recent studies with naive *Galleria mellonella*-apoLp-III gave first indications of an unexpected role of that protein in insect immune activation (Niere *et al.*, 1999). In this research, we cloned a cDNA encoding putative apoLp-III from the silkworm, *Bombyx mori* injected with *E. coli* and characterized.

Materials and Methods

Materials - Insect : *B. mori* Jam123

Library : *B. mori* cDNA library

Methods - Differential screening, nucleotide sequencing, BLASTx search through

GenBank database, phylogenetic analysis (PAUP version 4.0)

Total RNA isolation, Northern blot analysis

Results and Discussion

To identify novel genes that are expressed specifically or preferentially in immunized-*B. mori*, we constructed a cDNA library using whole bodies of *B. mori* larvae injected with *E. coli*, carried out the differential screening using cDNA synthesized with total RNA from *B. mori* injected with *E. coli* or not, respectively, and selected the up-regulated clones. Among these clones, we focused on the cDNA showing the significant similarity with apolipoprotein III from other insects, analyzed the nucleotide and deduced amino acid sequences. The pupative *B. mori* apoLp-III cDNA (GenBank Acc. No. AY341912) contained 1,131 bp encoding 186 amino acid residues. The *B. mori* Jam123 apoLp-III showed 99%, 98%, 99%, 65%, 64%, 59%, 63%, 15% and 9% nucleotide sequence identity to the *B. mori* P50, *B. mori* N4, *B. mandarina*, *M. sexta*, *S. litura*, *G. mellonella*, *E. postvittana*, *L. migratoria* and *A. domesticus*, respectively. Phylogenetic analysis revealed that the nucleotide and amino acid sequences of the *B. mori* apoLp-III cDNA formed a highly inclusive subgroup with Bombycidae. But, it was interesting that *B. mori* Jam 127 is closer to *B. mandarina* than *B. mori* P50 and *B. mori* N4. Northern blot analysis showed

a signal in the fat body, posterior silk gland and mid-gut.

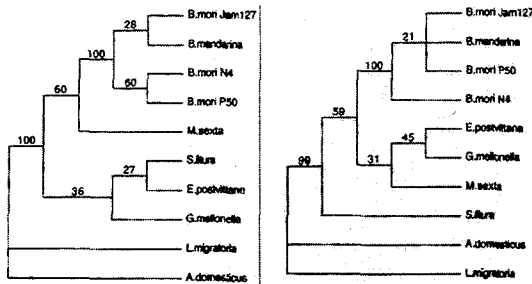


Fig.1. Relationships among nucleotide (left) and amino acid (right) sequences of the *B. mori* Jam123 apoLp-III and the known apoLp-IIIs.

References

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