

Molecular Cloning of a cDNA Encoding a Putative Larval and Pupal Cuticle Protein from the Chinese Oak Silkworm *Antheraea pernyi*

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Objectives

The insect cuticle undergoes drastic morphological alterations during postembryonic development and is an extracellular structure composed mainly of chitin and proteins that are synthesized and secreted by epidermal cells. Cuticle proteins, the major components of insect integument, are recently being focused as a model for studying the mechanisms of gene regulation during molting and metamorphosis.

A number of cuticle proteins have been identified and characterized in various insect species. Especially, larval cuticle proteins (LCPs) designated as LCP17, LCP18, LCP22 and LCP30 are well characterized in the silkworm, *Bombyx mori*. Biosynthesis of cuticle proteins is controlled stage-dependently and is regulated by the hormones ecdysteroid and juvenile hormone. However, no cuticle protein genes have been reported from the Chinese oak silkworm, *Antheraea pernyi*.

In order to obtain further genetic information on the cuticle protein from lepidopteran insect, we have cloned a cDNA encoding a larval and pupal cuticle protein homologue from the Chinese oak silkworm, *A. pernyi*. In this paper, the cloning, sequencing and characterization of a cDNA of *A. pernyi* larval and pupal cuticle protein homologue are described.

Materials and Methods

Materials - Chinese oak silkworm, *Antheraea pernyi*

Methods - cDNA library screening, nucleotide sequencing and data analysis, RNA isolation and Northern blot analysis

Results and Discussion

A cDNA encoding a putative larval and pupal cuticle protein was cloned from the Chinese oak silkworm, *Antheraea pernyi*. The cDNA sequence was 447 bp in length, encoding 149 amino acid residues. The predicted molecular mass for *A. pernyi* cuticle protein was approximately 16.4 kDa. The deduced amino acid sequence of the *A. pernyi* cuticle protein cDNA showed 43% identity to *Tenebrio molitor* cuticle protein and 42% to *Locusta migratoria* cuticle protein (Fig. 3). Northern blot analysis revealed that *A. pernyi* cuticle protein showed epidermis-specific expression. The expression profile of *A. pernyi* cuticle protein revealed by Northern blot analysis that the high-level mRNA expression of *A. pernyi* cuticle

protein was detected on the first day of larval ecdysis and on the first day after larval-pupal metamorphosis.

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-33                GCGAGCTTTAAGCGTTTGTACTGCTGGAG
1  ATGATGTTAAACTTCTTCTGTTTGTGGCCATGGTGGCAGTCCATGGCGAGCGCTT
1  H H L K L L L F C C A H U A U H G G A L
61  ATTTCTCCAGTGTACGGTCTCTTATAGCTATGGTCTTGGAAATCCATACAGCTCGTAT
21  I S P U Y G A P Y S Y G S W H P Y S S Y
121 CCATCAACACTGCATTGGCTTACAGCACTTCAACACGACAGGCTCTCCGTTAACTCA
41  P S T P A L A S Q H S H T Y R S P F H L
181 GGCAGGTATCTACATACTCGAAATCTGTTGACACCCCACTTCTACTGTGGGTAAAGCA
61  G Q U S T Y S K S U D T P F S S U R K A
241 GACATCGCGTTAGCAACCTTGGCGTTGGCAGTATCCCTGCTTACAGCGGTTTGGTCTGCT
81  D I R U S H P G U A U S P A Y S G F A A
301 CCATACGCTCTCATGTGCGTTTGGCCGCGCCACTAACAGCTCCCGTGGCTAAAGTTGCC
101 P Y U S H U G L A A P L T A P U A K U A
361 ACTGGACTCTTAGGTGTGGCATACTCAGCAGCTCTGCTGTGTCCACATGACATATACC
121 T G L L G U A Y S A A P A U S H H T Y T
421 AACGGACTCGGCCTTGCATATGCCTGGTAAATTACGCACGCAACGTCGATCATATTGAT
141 H G L G L A Y A W *
481 TCCATTGTATTGTTAAAGATTCTTTTTATTGTTACTCTGATAGTTGTTAACATTATTT
541 CATTACCTAACCTTACTTATATTACAAATAAATATTAAACTAAAAAAAAAAAAAAAAA

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Fig. 1. The nucleotide and deduced protein sequence of the *A. pernyi* cuticle protein cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. In the cDNA sequence, the polyadenylation sequence is underlined.

References

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