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Molecular Characterization of Silkworm Cathepsin D

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We have cloned a cDNA encoding the cathepsin D (EC 3.4.23), a member of the aspartic proteases, from the silkworm, *Bombyx mori*. The *B. mori* cathepsin D cDNA contains an open reading frame of 1,155 bp encoding 385 amino acid residues. Two catalytic aspartyl residues were conserved in the deduced amino acid sequences of *B. mori* cathepsin D at positions Asp 84 and 269. The deduced amino acid sequence of the *B. mori* cathepsin D cDNA was closest to the *Aedes aegypti* (63% protein sequence identity) and next to both *Aperiona germari* and *Drosophila melanogaster* (60% protein sequence identity), but low sequence identity (27%) to *Blattella germanica* cathepsin D. Phylogenetic analysis confirmed a closer relationship of the deduced amino acid sequences of the *B. mori* cathepsin D gene to the *A. aegypti*, *A. germari* and *D. melanogaster* within the insect cathepsin D group. The *B. mori* cathepsin D cDNA was expressed as a 40-kDa polypeptide in the baculovirus-infected insect Sf9 cells and *N*-glycosylation of the recombinant cathepsin D was revealed by tunicamycin to the recombinant virus-infected Sf9 cells, demonstrating that the silkworm cathepsin D is glycosylated. The expression profile of *B. mori* cathepsin D revealed by Northern blot and Western blot analyses that the high-level expression of *B. mori* cathepsin D was detected in fat body on the end of the fifth instar larvae and in midgut on the first day to third day of pupal stage, demonstrating that *B. mori* cathepsin D is differentially and spatially expressed in fat body and midgut with growth stage. To understand further functional roles of the cathepsin D in silkworm, we have elucidated the effects of reduced endogenous cathepsin D mRNA levels in infected silkworm via RNA interference (RNAi). The RNAi knock-down of cathepsin D resulted in the failure of silkworm larvae to complete the larval-pupal metamorphosis or in morphogenetic defects including abnormal pupae. The cathepsin D RNAi also prevented the deterioration of pupal gut. These results suggest that the *B. mori* cathepsin D is involved in both cellular remodeling associated with larval-pupal metamorphosis and gut deterioration during the pupal stage.