A10

Molecular Cloning and Characterization of Silkworm Cathepsin D

Zhong Zheng Gui, Kwang Sik Lee, Bo Yeon Kim, Hung Dae Sohn and Byung Rae Jin

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea

The *Bombyx mori* silkworm cathepsin D cDNA contains an open reading frame of 1,155 bp encoding 385 amino acid residues with two catalytic aspartyl residues at positions Asp 84 and 269. 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 understandfurther functional roles of the cathepsin D in silkworm, we have elucidated the effects of reduced endogenous cathepsin D mRNA levels in silkworm via RNA interference (RNAi). The RNAi-mediated cathepsin D reduction 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.