

cDNA Cloning and Developmental Expression of Hemolin Gene in *Bombyx mori*

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Objectives

1. To compare a full cDNA sequence of hemolin, a bacteria-induced protein of lepidopteran insects, from 4 geographically different strains of *Bombyx mori*.
2. To determine developmental profiles of hemolin gene expression in *Bombyx mori*.

Materials and Methods

Materials : Insect - *Bombyx mori* (4 geographical strains)

Bacteria - *Micrococcus lysodeikticus*

Methods : RNA preparation, RACE, RT-PCR, Cloning, Sequencing, Northern Hybridization

Results and Discussion

We isolated a 1.4 kb hemolin cDNA from a bacteria-injected fifth-instar larva and determined its nucleotide sequence. The analysis of sequence similarity was conducted between 4 geographical strains.

The tissue specific and developmental profile of the level of hemolin mRNA was determined in the absence of bacterial challenge. Hemolin mRNAs were detected in midgut and silk gland, but not in hemocytes, fat body, testis and ovary. During development of *B. mori* hemolin mRNAs in both midgut and silk gland were not detected until the spinning stage of the last instar larvae, however, dramatically increased after the completion of spinning and gradually decreased until pupal and adult stage. Thus, the role of hemolin is supposed to be unique for the physiological process at the post-spinning stage in *B. mori*.



Fig 1. Expression profile hemolin mRNA in the midgut of *B. mori*. **A** : Total RNA 10 μ g was loaded in each lane. 1-5, each larval instar, respectively (whole bodies were used for 1-3 larval instars); 6, after spinning; 7, white pupa; 8, pupa; 9, adults (σ); 10, adults (♀); 11, bacteria injected fifth instar. **B** : rRNA stained with ethidium bromide as a control.