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# Characterization of insect cytosolic juvenile hormone binding protein gene: Highly homology with vertebrate glyoxalase domain containing protein 4

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ABSTRACT

Cytosolic juvenile hormone binding protein (cJHBP) is a carrier of juvenile hormone (JH) in insects, however knowledge about its evolution and expression remains extremely limited. In this study, a gene encoding for cJHBP was isolated from the Chinese oak silkmoth *Antheraea pernyi*. A database search showed that the homologous sequences were present in several animal species including nematodes, insects, tunicates, fish, and mammals. The *A. pernyi* cJHBP had 54–85% identity with its homolog from other insects, and 58–62% identity with vertebrate glyoxalase domain containing protein 4 (Glod-4). Phylogenetic analysis supported the hypothesis that insect cJHBP shares a common ancestor with vertebrate Glod-4. RT-PCR detection showed that the *cJHBP* gene was expressed throughout the developmental stages and in all tested tissues of *A. pernyi*, which agreed with the data from *Bombyx mori cJHBP* and *Homo sapiens Glod-4*. These data suggest that insect cJHBP may play a similar function as vertebrate Glod-4.



## 1. Introduction

Juvenile hormone (JH) is a sesquiterpenoid molecule found in invertebrates and some plants (Yohann, 2005). JH regulates many critical aspects of insect physiology including development, metabolism, diapause and reproduction (Riddiford, 1985; Gilbert et al., 1996). JH also functions as an allelopathic agent in plants (Ji et al., 2007). JH may be bound by JH binding proteins (JHBP) to enable immobilisation, regulate degradation or enable transport (Orth et al., 2003).

Efforts have been directed toward the identification and characterization of insect JH receptors. The presence of hemolymph and intracellular JHBPs has been demonstrated in several insects, such as *Drosophila* (Chang et al., 1980; Klages et al., 1980), *Galleria mellonella* (Wisniewski et al., 1988), *Manduca sexta* (Orth et al., 2003), *Bombyx mori* (Suzuki et al., 2011), and *Pararge aegeria* (Carter et al., 2013). Intracellular JHBPs, including nuclear JHBP (nJHBP) and cytosolic JHBP (cJHBP), are

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considered to be the JH receptors (Wang et al., 1989; Shemshedini et al., 1990; Konopova and Jindra, 2007). The cJHBP was first found in *Drosophila melanogaster* (Chang et al., 1980), subsequently confirmed by using labeled JH III (Chang et al., 1985; Konopova and Jindra, 2007), and plays a role in transport in intracellular compartments (Yohann, 2005). Hemolymph JHBPs (hJHBPs) and nJHBPs have been characterized in different insect groups (Lerro and Prestwich, 1990; Fujimoto et al., 2013), but knowledge on cJHBP remains extremely limited, especially its evolution.

In the present study, we have identified a putative gene coding for cJHBP from the Chinese oak silkmoth, *Antheraea pernyi* (Lepidoptera: Saturniidae), one of the most well-known wild silkmoths used for silk production, and also as a high-quality protein food (Liu et al., 2010; Peigler, 2012). Sequence alignments reveal that cJHBP and its homologs are relatively highly conserved throughout evolution of animals. Our results suggest that insect cJHBP shares a common ancestor and may function in a similar manner as human glyoxalase domain containing protein 4 (Glod-4).

## 2. Materials and methods

### 2.1. Materials

The larvae of *A. pernyi* strain Shenhuang No. 2 were reared routinely on oak trees at the silkworm field of Shenyang Agricultural University. To detect the gene expression pattern, four developmental stages (eggs at day 5, fifth instar larvae, pupae, adults) and ten tissues of fifth instar larvae at day 10 (blood, fat body, midgut, silk glands, integument, Malpighian tubules, spermaries, ovaries, brain, muscle) were sampled and then stored at −80 °C. To examine the effect of heat stress, eggs at day 6 were treated at 50 °C for 20 s and then incubated at 25 °C. These treated eggs can develop and hatch as the control eggs. After 1 h and 3 h of treatment, the whole eggs were frozen in liquid nitrogen and stored.

### 2.2. Gene isolation and PCR primer

A full-length cDNA library of *A. pernyi* was constructed (Li et al., 2009). Through EST sequencing, an EST encoding cJHBP homologue was isolated, and then the clone carrying the cDNA insert was used to complete the full-length cDNA sequence of the *A. pernyi cJHBP* gene (GenBank accession no. KF830718). The primer pair ORF-F and ORF-R was used to amplify the entire open reading frame (ORF) of this gene (Table 1).

### 2.3. RNA extraction, cDNA synthesis and RT-PCR

Total RNA was extracted with a RNAprep Pure Tissue Kit (TIANGEN Biotech, Beijing, China) according to the manufacture's instructions. The first-strand cDNA was generated with 2 ug of total RNA by a TIANScript RT kit (TIANGEN Biotech). The *eIF4A* gene was used as the internal control with the specific primer pair eIF4A-F and eIF4A-R (Chen et al., 2013). RT-PCR reactions were performed on a Bio-Rad S1000 thermal cycler (Bio-Rad Laboratories, Inc.), with the following cycles: initial denaturation at 95 °C for 5 min; followed by 30 cycles of 1 min at 95 °C, 30 s annealing at 54 °C, 30 s extension at 72 °C; and a final extension at 72 °C for 10 min. The PCR products were purified from the gel and sequenced directly to ensure the specificity.

### 2.4. Quantitative RT-PCR analysis

For qRT-PCR analysis, the specific primer pair JHBP-F and JHBP-R were used, which were designed using Beacon Designer 7.0 software (Premier Biosoft International). The qRT-PCR was carried out using a Roche Light Cycler 480 (Hoffmann-La Roche Ltd.), with the following cycles: initial denaturation at 95 °C for 2 min; followed by 40 cycles of 15 s at 95 °C, 30 s annealing at 60 °C, 30 s extension at 68 °C; followed by a stage of 60–95 °C determine melting curves of the amplified products. The relative changes for gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The qRT-PCRs were performed three parallel measurements for each cDNA samples from independent RNA extraction. Statistical analysis was performed with SPSS 16.0. A two-tailed Student's test was used to determine the difference between the groups and $P < 0.01$ was considered as significance.

**Table 1**
Primers for RT-PCRs used in this study.

| Gene | Primer pairs and sequences (5′–3′) | PCR product (bp) |
|---|---|---|
| Full ORF | | |
| *cJHBP* | F: ATGAA GATTA ACGGA CGCGC C<br>R: TTAGG CTCGA GATTT ATCTG C | 858 |
| qRT-PCR | | |
| *cJHBP* | F: GGCGG TTACA AGTTC TAC<br>R: GTTCC AGTAT GCGAT TGAT | 111 |
| *eIF-4A* | F: TCCTC TCGTG TGCTT ATC<br>R: CCACC TCTTC CGATT CTAT | 128 |