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Juvenile hormone esterase – a key enzyme for host-parasitoid interactions

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Abstract: Juvenilhormon-Esterase, ein Schlüsselenzym in Wirt-Parasitoid-Interaktionen.

Die gregäre, parasitische Brackwespe *Glyptapanteles liparidis* (Hym., Braconidae) entwickelt sich in den Raupen des Schwammspinners *Lymantria dispar* (Lep., Lymantriidae). Bei der Eiablage injiziert die Wespe Viruspartikel in die Leibeshöhle der Wirtsraupe, die deren Immunabwehr lahm legen und so das Einkapseln der Eier verhindern. Im Laufe der Entwicklung der Parasitoidenlarven ändern sich die Juvenilhormongehalte in der Wirtshämolymphe dramatisch. Das für den Abbau des Juvenilhormons verantwortliche Enzym, die Juvenilhormon-Esterase (JH-Esterase), wird dramatisch herunterreguliert, während der Titer an Juvenilhormon steigt und kurz vor dem Ausbohren der Parasitoiden das Hundertfache des Normalwertes von unparasitierten Raupen erreicht. Wir konnten in unseren Untersuchungen nachweisen, dass die Polydnaviren für die Blockierung dieses spezifischen Enzyms verantwortlich sind, wobei aber die Aktivität des JH-Esterase Gens in den parasitierten Raupen unverändert bleibt. Mit der Häutung der Parasitoidenlarven in das 2. Larvenstadium beginnt der Hormonspiegel in der Hämolymphe kontinuierlich anzuwachsen. Auffallend dabei ist, dass davon vor allem das JH III Homolog betroffen ist, welches als das bisher einzige von Hymenopteren produzierte Homolog bekannt ist. Mit Hilfe von Inkubationsversuchen konnten wir zeigen, dass dieses Homolog von den Parasitoidenlarven selbst produziert und in die Wirtsraupe abgegeben wird. Die Syntheseleistung und die Abgabe von JH durch die Corpora allata der Wirtsraupe wird durch die Parasitierung dagegen nicht gesteigert; der im Vergleich zu JH III geringere Anstieg von JH II – dem dominierenden Homologon in Lepidopteren – ist offenbar auf die unterdrückte Enzymaktivität zurückzuführen. Aus den vorliegenden Ergebnissen wird klar, dass Parasitoide und Polydnaviren auf unterschiedlichen Ebenen in die Hormonregulation eingreifen und erst das komplexe Zusammenspiel beider eine erfolgreiche Parasitierung ermöglicht.

Key words: juvenile hormone esterase, host parasitoid interactions, *Lymantria dispar*, *Glyptapanteles liparidis*

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Hymenopteran endoparasitoids that develop inside their lepidopteran host may exert a multitude of interactions with their host until they are able to emerge successfully from a developmentally arrested host that finally dies. Parasitoid interferences comprise physiological and biochemical modifications in the host endocrine and immune system which in turn affect host growth and development (reviewed in EDWARDS & WEAVER, 2001).

We use the gypsy moth, *Lymantria dispar* (Lep., Lymantriidae) and the endoparasitic, polydnavirus (PDV)-carrying braconid wasp *Glyptapanteles liparidis* (Hym., Braconidae) as a model system to study the endocrine changes associated with parasitism. Following wasp oviposition into young gypsy moth larvae, the parasitoids develop through two endoparasitic instars, and then emerge as newly molted third instars from a host that dies in the larval stage. In previous studies we have already described the endocrine changes in parasitized gypsy moth larvae which show an increase in juvenile hormone (JH) titers, a shift from JH II to JH III as the dominant homologue, and a prominent decrease in the JH degrading enzymes (SCHOPF & al., 1996;

SCHAFELLNER & al., 2004). Here, we investigated the possible mechanisms that account for the JH elevating effects such as (i) stimulated host corpora allata activity, (ii) reduced activity of the JH metabolic enzymes such as JH esterase, and (iii) synthesis and release of JH by the parasitoid larvae.

Material and Methods

Insects. Gypsy moth larvae were reared on wheat germ diet at 20±1°C, ~70% R.H. and a photoperiod of LD 16:8 h. Larvae were parasitized or so-called pseudoparasitized by *G. liparidis* females in premolt to third instar. Under the experimental conditions applied, parasitoid larvae hatched 4 days post parasitization (dpp), molted into second instars 11 dpp and emerged as newly molted third instars from a developmentally arrested host larva appr. 17 dpp. Parasitoids egressed from fourth-instar hosts when parasitized in premolt to the third instar. For pseudoparasitization we used gamma-sterilized wasps that inject fully functional PDV and venom, but no viable eggs (TILLINGER & al., 2004). Hemolymph and tissue samples were collected throughout the host's fourth instar.

JH titers. Hemolymph samples and whole body extracts of parasitoids were analyzed for JHs by gas chromatography-mass spectrometry according to the method of REMBOLD & LACKNER (1985). For detail see SCHAFELLNER & al. (2004).

JH esterase activity assay. JH esterase activity in hemolymph and fat body homogenates was measured using the partition assay of HAMMOCK & SPARKS (1977), with labelled JH III as substrate for JHE. After organic extraction, the JH metabolites are partitioned into an aqueous phase and the radioactivity is determined by liquid scintillation counting.

JH esterase gene expression. Primers specific for the *L. dispar* JH esterase encoding gene were developed from a partial cDNA library (NUSSBAUMER & al., 2000). Relative quantitation of gene expression was achieved by total RNA extraction from larval fat body homogenates and subsequent real-time RT-PCR with Sybr Green using an ABI PRISM® 7700 Sequence Detection System. 18S rRNA was used as an internal control.

Corpora allata activity. A radiochemical assay with L-[methyl-14C] methionine as radiotracer was employed to assess in vitro rates of JH biosynthesis in unparasitized and parasitized fourth instar gypsy moth larvae (SCHAFELLNER & al., 2004).

Results and Discussion

JH levels in hemolymph. Hemolymph JH titers, especially the JH III levels, increased dramatically when gypsy moth larvae were parasitized by *G. liparidis* (Fig. 1). This rise in hormone concentrations coincided with the parasitoids' molt to second instars and could not be observed in pseudoparasitized hosts which contain polydnavirus (PDV), but no parasitoids. The extraordinarily high JH titers prior to parasitoid emergence are obviously caused by a substantial release of JH from second instar parasitoids (SCHAFELLNER & al., 2004). JH titers dropped to very low levels in the post-emergence period (Fig. 1).

JH metabolism. JH esterase is the only relevant JH degrading enzyme present in hemolymph and fat body of gypsy moth larvae (Schafellner, unpublished). Unparasitized larvae displayed two activity peaks, one on day 2 and the other, higher one on day 6 of the fourth instar (Fig. 2a). In hosts parasitized by *G. liparidis*, enzyme activity was dramatically reduced throughout the instar and no peak activities were observed (Fig. 2a); enzyme down-regulation was maintained throughout the course of parasitoid development and during the post-emergence period. Similarly, JH esterase action was depressed following pseudoparasitization (Fig. 2a), but host larvae molted and pupated.

JH esterase activity in the fat body of unparasitized fourth-instar larvae was similar to the pattern observed in hemolymph (Fig. 2b). Fat body from parasitized larvae showed no enzyme activity peaks and little daily variation; levels were generally lower except during mid-instar (Fig. 2b).

JH esterase gene expression. JH esterase gene expression was highest on days 2 and 6 of the fourth instar and low during mid-instar. JH esterase activity in host fat body and JH esterase gene activity fluctuated in parallel with each other (Figs. 2 and 3), confirming the assumption that the larval fat body is the major source of hemolymph JH esterase (WROBLEWSKI & al., 1990). Parasitism had no influence on JH esterase gene activity. Transcription levels of mRNA were similar in both unparasitized and parasitized gypsy moth larvae (Fig. 3). This finding gives evidence to the fact that JH esterase suppression might be due to post transcriptional/translational modifications rather than to a down-regulation of gene transcription.

JH synthesis of host CA and parasitoids. JH synthesis and release by host corpora allata was not up-regulated in parasitized larvae (Fig. 4). Results from our *in vitro* study on total JH biosynthesis of CA excised from parasitized larvae showed no stimulation, but lower rates of biosynthesis than with glands from unparasitized insects. Nevertheless, it cannot be excluded that the results merely reflect increased metabolic deactivation of synthesized JH (i.e. by JH degrading enzymes), rather than reduced biosynthesis *per se*.

When incubated into insect culture medium, eggs of *G. liparidis* and 1st instar parasitoids did not release JH. Second instars, however, added significantly to elevated host hormone levels by producing and secreting JH (Fig. 5). After emergence from host larvae, JH synthesis of third instar parasitoids ceased (Fig. 5). JH III was the only homolog detected in parasitoid homogenates (Schafellner & al., 2004).

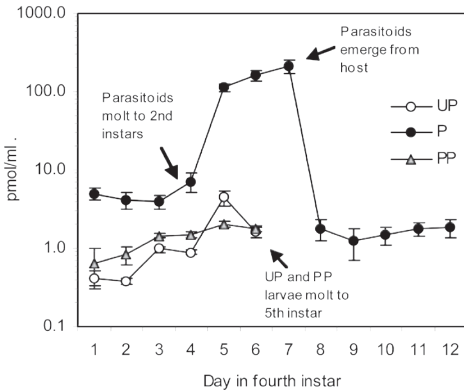

Figure 1. JH III titers (mean±SE) in hemolymph of unparasitized (UP), parasitized (P), and pseudo-parasitized (PP) gypsy moth larvae (n=11-17 for each day and treatment).

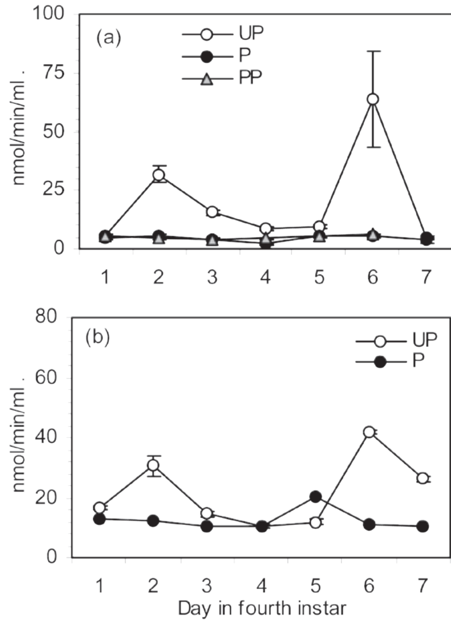

Figure 2. JH esterase enzyme activity (mean±SE) in (a) hemolymph and (b) fat body of unparasitized (UP) parasitized (P), and pseudoparasitized (PP) gypsy moth larvae (n=10-14 for each day and treatment).

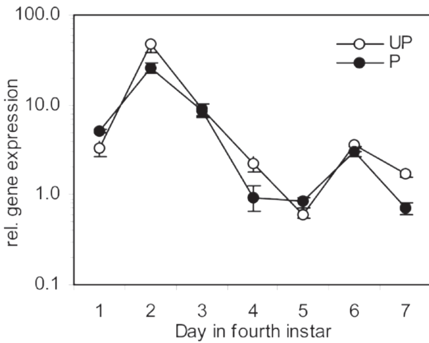

Figure 3. JH esterase gene activity (mean±SE) in the fat body of unparasitized (UP) and parasitized (P) gypsy moth larvae (n=10-15 for each day and treatment) .

Our data indicate that *G. liparidis* has evolved several strategies to create a JH milieu that prevents further host molts. Wasp PDV/venom components act as a repressor of JH esterase enzyme activity. Enzyme activity is blocked in both host hemolymph and fat body. The JH esterase gene expression in host fat body, however, is not repressed; posttranslational modifications of the protein are likely to be responsible for the observed enzyme suppression. The JH titers in hemolymph increase due to JH esterase inhibition, but the parasitoid larvae release JH into host hemolymph and elevate specifically JH III levels.

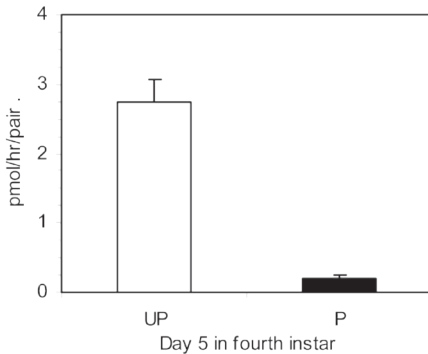

Figure 4. JH release (mean±SE) by corpora allata of unparasitized (UP, n=32) and parasitized (P, n=23) gypsy moth larvae.

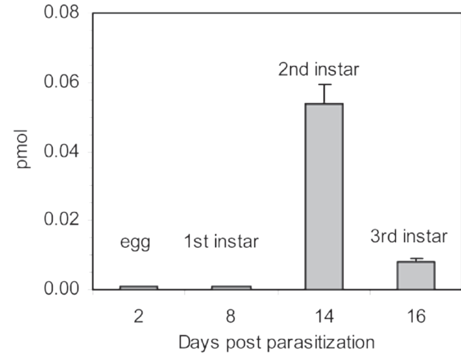

Figure 5. JH III release (mean±SE) by *G. liparidis* eggs, 1st, 2nd, and 3rd (newly emerged from host) instars. For each sample (n=8-14), 50 eggs, 40 1st instar, and 10 2nd or 3rd instar parasitoids were pooled.

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