Disruption of Lepidopteran Metamorphosis By Polydnaviruses

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Summary

It is common for endoparasitic wasps to suppress metamorphosis in their lepidopteran hosts. This developmental pathology is usually induced by wasp-borne factors injected into the caterpillar during oviposition. Our laboratory focuses on the effects of a group of dsDNA viruses known as polydnaviruses (PDVs) that replicate in the ovaries of certain ichneumonid and braconid wasps. PDV gene products are believed to be responsible for perturbations of the host endocrine events governing metamorphosis. Here, we briefly review our work on the suppression of *Choristoneura fumiferana* metamorphosis by the PDV of *Tranosema rostrale* (TrIV), including data on the hormonal correlates of the larval-pupal transformation, as well as on TrIV gene expression and cloning. We also present new data on the effects of parasitism by *T. rostrale* on *C. fumiferana* hemolymph protein titers.

Introduction

Polydnaviruses (PDV) form a group of unusual viral entities that feature segmented dsDNA genomes. They comprise two genera, *Ichnovirus* (IV) and *Bracovirus* (BV), each associated with parasitic wasps belonging to selected ichneumonid and braconid subfamilies,

respectively. PDVs replicate in the ovarian calyx tissue and accumulate in the lumen of the wasp ovary where they form the particulate fraction of the calyx fluid (Cxf). During oviposition, Cxf is injected along with eggs into the caterpillar host, where viral genes are expressed. PDV gene products are believed to be responsible for perturbations of host physiology, including immune dysfunction and disruption of the endocrine events associated with metamorphosis, both apparently beneficial to the developing wasp larva (Stoltz, 1993).

The ichneumonid wasp *Tranosema rostrale* transmits an ichnovirus (TrIV) to its host, *Choristoneura fumiferana*, during oviposition. Given the uncommonly weak impact of TrIV on the cellular immune response of its lepidopteran host (Doucet & Cusson, 1996a), this system seems well suited to the identification of PDV genes involved in developmental disruption.

Parasitization of newly molted 5th- or 6th-(last) instar *C. fumiferana* larvae by *T. rostrale* causes developmental arrest of the last instar, effectively suppressing its metamorphosis. Similarly, injection of *T. rostrale* Cxf into healthy larvae causes a dose-dependent delay in the initiation of the larval-pupal transformation, an effect that can be abolished by prior treatment of Cxf with a DNA cross-linker (Doucet & Cusson, 1996b).

The direct cause of the observed developmental arrest is an inhibition of the normal rise in 20-hydroxyecdysone (20E) titers, observed in both parasitized and Cxf-injected last-instar larvae. Whether TrIV gene products have a direct effect on the biosynthetic activity of the prothoracic glands (PTG) or whether the virus infects PTG cells remains unclear. Interestingly, the activity of juvenile hormone esterase (JHE) is strongly inhibited by both parasitism and Cxf injection, but this inhibition does not have a measurable impact on the JH titer of parasitized larvae. Likewise, the post-molt decline in rates of JH biosynthesis, as measured *in vitro*, is not affected by either parasitization or Cxf injection at the beginning of the last instar. These observations call into question the functional significance of TrIV-induced JHE inhibition as well as the role of JHE in the initiation of metamorphosis (Cusson *et al.*, 2000 and in press).

Northern analysis of TrIV-specific RNAs from Cxf-injected 6th-instar larvae, using viral DNA as a probe, led to the identification of a dominantly transcribed 650-bp message, the temporal pattern of which coincided with the induction of developmental arrest (Béliveau *et al.*, 2000). The corresponding gene was cloned (*TrV1*) and found

to encode a small 103-amino-acid protein showing no similarity to any other known protein except the VHv1.4 protein, a gene product of the ichnovirus of *Campoletis sonorensis* (Cui & Webb, 1996) that shows limited similarity to TrV1 within the first ~45 amino acids of the N-terminal sequence. Interestingly, both northern and western tissue-specific analyses of TrV1 expression indicated that fat body and epidermis constitute the principal tissues of TrV1 expression, while little or no expression could be detected in the hemocytes (Béliveau *et al.*, 2000), considered an important site of transcription for several PDV genes involved in immune suppression (Webb, 1998).

Three additional TrIV genes have recently been cloned, including one (*TrV2*) that encodes a protein showing a high degree of similarity to TrV1 and the transcript of which may be difficult to distinguish from that of *TrV1* in northern analysis (Cusson *et al.*, in press).

Our current work is aimed at identifying TrIV gene products that are responsible for the disruption of the hormonal events leading to host metamorphosis, as well as characterizing the cellular/molecular mechanisms effecting such disruption. Little is known about the mechanisms through which PDVs block metamorphosis except, perhaps, for the documented post-transcriptional inhibition of certain development-associated proteins such as JHE and storage proteins (e.g. Shelby & Webb, 1994, 1997). We have previously reported the absence of transcriptional inhibition of *C. fumiferana* JHE (CfJHE) in Cxf-injected larvae; given that JHE activity (and presumably titer) is suppressed in these insects, a translational inhibition of CfJHE by TrIV has been suggested (Béliveau *et al.*, 2000). Here, we present data on the effect of parasitism by *T. rostrale* on the levels of *C. fumiferana* hemolymph proteins, including those of two storage proteins characterized earlier by Palli *et al.* (1998).

Materials and Methods

Insect rearing and parasitization. *C. fumiferana* larvae and *T. rostrale* wasps were obtained as described previously (Doucet and Cusson, 1996a, b), and parasitization was carried out as described in Béliveau *et al.* (2000).

Isolation and analysis of hemolymph (HL) proteins. HL samples were collected from the punctured prolegs of 6th-instar *C. fumiferana* larvae at 4 h, and 1, 2, 3, 4, 5, 6 and 8 d post parasitization (p.p.; larvae parasitized 1 d after the molt to the 6th instar) and from healthy

caterpillars of equivalent ages, the oldest group excepted (all healthy larvae had pupated by then). To 5 µl of HL, 35 µl of PBS (phosphate-buffered saline) was added followed by a 10-min centrifugation at 12,000 *g*, 4°C, to remove hemocytes. The supernatants were snap-frozen in liquid nitrogen and stored at –80°C until processed.

We first ascertained TrIV infection of each insect from which HL had been drawn by conducting a western analysis of TrV1 expression in individual samples. To this end, a volume of supernatant equivalent to 1 µl of HL was separated on a 7.7% SDS-PAGE gel (Khalkhali-Ellis, 1995), and the proteins were electrotransferred onto Hybond-P (Amersham Pharmacia Biotech). Immunodetection was carried out according to the membrane manufacturer's instructions using 1% BSA in TBST (Tris-buffered saline and 0.05% Tween-20) as the blocking solution. A 1:500 dilution of the TrV1-His antiserum (Béliveau *et al.*, 2000) was used as the primary antibody. TrV1 was detected using a 1:30,000 dilution of the goat anti-rabbit IgG antibody conjugated with alkaline phosphatase (Sigma) and the Alkaline Phosphate Conjugate Substrate kit (Bio-Rad). Samples from which a TrV1 signal could be detected were pooled (N = 2-4) and submitted to a new western analysis. For electrophoretic analysis of proteins, a volume of supernatant equivalent to 0.05 µl of HL was separated on a 7.5% SDS-PAGE gel (Laemmli, 1970); the latter was stained using the Silver Stain Plus kit (Bio-Rad).

Results

The temporal pattern of TrV1 levels in the HL of parasitized larvae was similar to that observed for *TrV1* transcription, by northern analysis, although the intensity of the signal observed on the western blot shown here (Fig. 1, upper panel) was more variable after 3 d p.p. than that seen on the northern blot (Béliveau *et al.*, 2000); this difference may be due to variations in the dose of virus injected by the wasp during oviposition.

With the exception of the 1 d p.p. time point, overall protein titers were higher in healthy than in parasitized larvae (Fig. 1, lower panel). However, parasitism seemed to affect the titer of specific proteins while leaving others unaffected. Among the proteins whose titer was suppressed by parasitism, the most striking are CfDAP1 and CfDAP2, two hexamerin storage proteins produced by *C. fumiferana* last-instar larvae. This effect was already apparent before hatching of the wasp

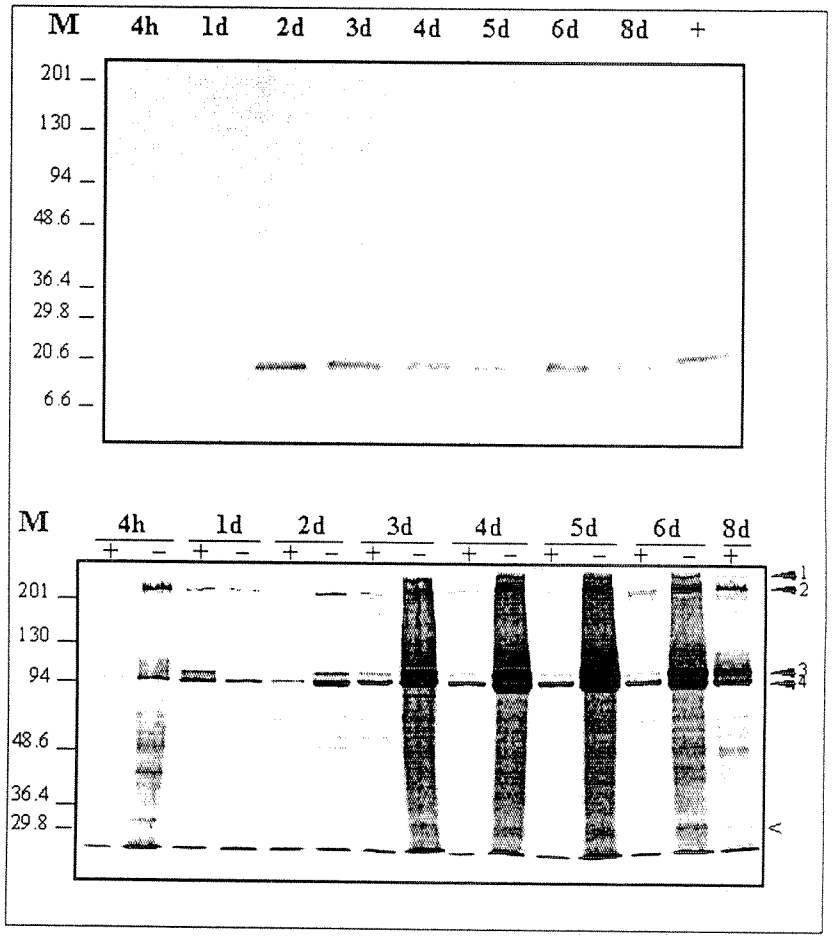

Figure 1. Upper panel: western analysis of TrV1 levels in the hemolymph of 6th-instar C. fumiferana *larvae at various times following parasitization (24 h after the molt) by* T. rostrale. *The "+" lane shows the recombinant His-tagged protein produced using a baculovirus expression system (Gibco BRL); lower panel: SDS polyacrylamide gel electrophoresis of proteins from the hemolymph of parasitized ("+"; same samples as those used for the western analysis shown in a) and healthy ("–") 6th-instar* C. fumiferana *larvae (see text for details). 1: unidentified protein; 2: apo-lipophorin I; 3: CfDAP1; 4: CfDAP2; <: protein whose titer seemed little affected by parasitism. The identity of CfDAP1 and CfDAP2 was confirmed by western analysis using a CfDAP-specific antiserum (Palli* et al., *1998). M: prestained SDS-PAGE broad-range MW standards (kDa) (BioRad).*

egg, 2 and 3 d p.p., suggesting that it is due to TrIV infection as opposed to the feeding activity of the parasitoid larva. Interestingly, titers of CfDAP1 seemed more strongly suppressed than those of CfDAP2. Two high-MW proteins were also differentially affected by parasitism; the band with the greater mobility is believed to be apo-lipophorin I, based on an SDS-PAGE analysis of a lipoprotein eluted from a native gel (Cusson, unpublished), while the identity of the other is unknown. Although a comparison with control HL is not possible here for the 8 d p.p time point, it can be seen that protein titers had substantially increased in this sample relative to those observed on earlier time points, perhaps as a consequence of reduced TrIV gene expression (see Fig. 1, upper panel, and Béliveau *et al.*, 2000). No parasitism-specific protein could be detected by simple silver staining of the SDS-PAGE gel.

Discussion

As shown previously for other host-parasitoid systems (e.g. Beckage and Kanost, 1993; Shelby and Webb, 1994, 1997), parasitism of *C. fumiferana* last-instar larvae by *T. rostrale* resulted in a significant alteration of the host hemolymph protein profile. Most striking was the suppression of the normal increase in the titer of the storage proteins CfDAP1 and CfDAP2, an effect already noticeable 2 days after parasitization, a time-point coinciding with the attainment of a high TrV1 expression level. Given that *T. rostrale* eggs do not hatch until the end of the third day following oviposition (Cusson *et al.*, 1998), the observed effect is likely attributable to TrIV, although this hypothesis needs to be verified experimentally. On day 1 p.p., however, the observed protein pattern seemed to be at odds with that seen on the following days; this apparent discrepancy could be due to a small difference in the developmental age of the two groups of larvae (the parasitized ones being slightly more advanced), combined with the absence of viral effect at that time, as suggested by the low level of TrV1 expression.

The suppressive effect of parasitism on the level of apo-lipophorin I, most noticeable on day 2 p.p., is in contrast with the lack of effect of parasitism by *C. sonorensis* and *Cotesia congregata*, and/or injection of their PDV, on the titer of this protein in their respective hosts (Beckage and Kanost, 1993; Shelby and Webb, 1994, 1997). Whether this observation reflects differences in the mode of action of PDV

gene products needs to be addressed. Clearly, however, the titer of other less abundant proteins was not affected by parasitism (see for example arrowhead on Fig. 1), suggesting the existence of a mechanism allowing selective inhibition of specific host proteins.

We are now assessing the effect of TrIV infection on the transcription of CfDAP1 and CfDAP2.

References

BECKAGE, N.E. and M.R. KANOST. Effects of parasitism by the braconid wasp *Cotesia congregata* on host hemolymph proteins of the tobacco hornworm, *Manduca sexta*. *Insect Biochem. Mol. Biol.* 23, 643-653 (1993).

BÉLIVEAU, C., M. LAFORGE, M. CUSSON and G. BELLEMARE. Expression of a *Tranosema rostrale* polydnavirus gene in the spruce budworm *Choristoneura fumiferana*. *J. Gen. Virol.* 81, 1871-1880 (2000).

CUI, L. and B.A. WEBB. Isolation and characterization of a member of the cysteine-rich gene family from *Campoletis sonorensis* polydnavirus. *J. Gen. Virol.* 77, 797-809 (1996).

CUSSON, M., C. BÉLIVEAU, M. LAFORGE, G. BELLEMARE, A. LEVASSEUR and D. STOLTZ. Hormonal alterations and molecular mechanisms underlying the induction of host developmental arrest by endoparasitic wasps, In: *Endocrine Interactions of Insect Parasites and Pathogens*. J.P. Edwards and R.J. Weaver, eds., BIOS Scientific Publishers, Oxford (in press).

CUSSON, M., C. LUCAROTTI, D. STOLTZ, P. KRELL and D. DOUCET. A polydnavirus from the spruce budworm parasitoid, *Tranosema rostrale* (Ichneumonidae). *J. Invert. Pathol.* 72, 50-56 (1998).

CUSSON, M., M. LAFORGE, D. MILLER, C. CLOUTIER and D. STOLTZ. Functional significance of parasitism-induced suppression of juvenile hormone esterase activity in developmentally delayed *Choristoneura fumiferana* larvae. *Gen. Comp. Endocrinol.* 117, 343-354 (2000).

DOUCET, D. and M. CUSSON. Role of calyx fluid in alterations of immunity in *Choristoneura fumiferana* larvae parasitized by *Tranosema rostrale*. *Comp. Biochem. Physiol.* 114A, 311-317 (1996a).

DOUCET, D. and M. CUSSON. Alteration of developmental rate and growth of *Choristoneura fumiferana* parasitized by *Tranosema rostrale*: role of the calyx fluid. *Entomol. Exp. Appl.* 81, 21-30 (1996b).

KHALKHALI-ELLIS, Z. An improved SDS-polyacrylamide gel electrophoresis for resolution of peptides in the range of 3.5-200 kDa. *Prep. Biochem.* 25, 1-9 (1995).

LAEMMLI, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685 (1970).

PALLI, S.R., T.R. LADD, A.R. RICCI, M. PRIMAVERA, I.N. MUNGRUE, A.S.D. PANG and A. RETNAKARAN. Synthesis of the same two proteins prior to larval diapause and pupation in the spruce budworm, *Choristoneura fumiferana*. *J. Insect Physiol.* 44, 509-524 (1998).

SHELBY, K.S. and B.A. WEBB. Polydnavirus infection inhibits synthesis of an insect plasma protein, arylphorin. *J. Gen. Virol.* 75, 2285-2292 (1994).

SHELBY, K.S. and B.A. WEBB. Polydnavirus infection inhibits translation of specific growth-associated host proteins. *Insect Biochem. Mol. Biol.* 27, 263-270 (1997).

STOLTZ, D.B. The polydnavirus life cycle. In: *Parasites and Pathogens of Insects* (eds N.E. Beckage, S.N. Thompson and B.A. Federici), Vol. I: Parasites. Academic Press, London, pp. 167-187 (1993).

WEBB, B.A. Polydnavirus biology, genome structure, and evolution. In: *The Insect Viruses* (eds L.K. Miller and L.A. Ball), Plenum, New York, pp. 105-139 (1998).

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