Polydnavirus infection inhibits synthesis of an insect plasma protein, arylphorin

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The wasp Campoletis sonorensis injects a segmented, double-stranded DNA polydnavirus (CsPDV) along with its egg during parasitization of Heliothis virescens larvae. After parasitization, CsPDV protects the wasp egg and larva by selectively disabling the host's cellular immune response. Other host physiological systems including growth and development are affected to the apparent benefit of the parasite. To begin the characterization of the biochemical effects and mode of action of CsPDV on host growth, the titre of a developmentally regulated insect storage protein, arylphorin, was studied. Parasitized or virus-infected insects had substantially less circulating arylphorin than control insects. Fat bodies from parasitized larvae also synthesized less arylphorin in vitro. However, Northern blots of total RNA from parasitized and non-parasitized, control insects showed that the arylphorin transcript level was unaffected by parasitization suggesting a biochemical block at the translational level. In vitro translation followed by immunoprecipitation of arylphorin indicated that the mRNA was present and translatable at equal levels in both parasitized and control insects. Injection of purified virus elicited the response observed in naturally parasitized larvae, demonstrating that the effect on arylphorin synthesis is mediated, either directly or indirectly, by polydnavirus gene product(s).

Introduction

Some parasitic wasps carry integrated, segmented, dsDNA viruses known as polydnaviruses. Polydnaviruses replicate only in the calyx cells of the female reproductive tract, bud into the oviduct lumen and are injected, along with the wasp egg, venom and ovarian proteins, into host insects during parasitization (for reviews see Fleming, 1992; Fleming & Krell, 1993; Stoltz & Whitfield, 1992; Beckage, 1993). The Campoletis sonorensis polydnavirus (CsPDV) has been characterized to the greatest extent at the molecular level. The CsPDV genome is composed of at least 28 DNA segments ranging in size from 6 to 21 kbp (Krell et al., 1982; Fleming, 1992). CsPDV replication has not been detected in parasitized lepidopteran insect larvae, although at least 12 viral mRNAs are expressed from several different DNA segments (Blissard et al., 1986; Theilmann & Summers, 1988).

The eggs from parasitic wasps are encapsulated by the host's cellular immune system when injected without their associated polydnavirus or along with inactivated virus (Edson et al., 1981). Proteins from the female reproductive tract are secreted into the oviduct lumen and are introduced into host larvae during oviposition. Some of these proteins bind to insect immunocytes (haemocytes) and may affect encapsulation directly (Webb & Luckhart, 1994). CsPDV is thought to act in conjunction with these ovarian proteins to block the cellular immune response to parasite eggs both rapidly and throughout the course of parasitization (Webb & Luckhart, 1994). Recent studies demonstrate that the M, 30K VHv1.1 protein also interacts directly with the immunocytes, granulocytes and plasmatocytes that mediate the insect encapsulation response (X. Li & B. A. Webb, unpublished results).

Other physiological effects of CsPDV on Heliothis virescens have been less studied but include the inhibition of host growth, delayed moulting and prevention of metamorphosis (Fleming, 1992; Vinson, 1990). Both natural parasitization and injection of purified CsPDV into fifth instar larvae result in degeneration of the prothoracic gland, the organ which synthesizes the steroid moulting hormone, ecdysone (Dover et al., 1988). This cytotoxic effect on the prothoracic gland may represent a mechanism through which polydnaviruses act directly on endocrine cells to regulate host development, although it was later determined that the effect is not evident in parasitized third or fourth instar larvae (Dover & Vinson, 1990). A number of new proteins are evident in the haemolymph of H. virescens after parasitization or CsPDV injection and the presence of a 50K to 55K protein (fuzzy band) has been correlated with successful parasitization in permissive hosts (Cook...
may constitute 70 to 80% of the total plasma protein in
Pan, 1978; Kanost has a high content of aromatic amino acids (Wyatt &
tivities is that biochemical assays for the physiological
insect growth. Arylphorin is a high Mr (approx. 450K)
removed from circulation by endocytosis into protein granules in
the penultimate larval instar (LeClerc & Miller, 1990). In

Fig. 1. (a) Parasitization of premoult penultimate instar larval H. virescens prevents the normal rapid increase in haemolymph arylphorin
titre. Coomassie blue-stained haemolymph proteins secreted from
unparasitized control (U) and parasitized (P) larvae 24 h p.p. (30 µg/m). By 48 h p.p. the large increase seen in unparasitized controls (U) is completely inhibited in parasitized animals (P). Protein standards are shown on the right. (b) The effect of injecting 0.01 female equivalents of calyx fluid on the haemolymph titre of arylphorin at 48 h post-injection. (c) The effect of injecting 0.1 female equivalents of CsPDV on the haemolymph titre of arylphorin. Haemolymph samples were taken 48 h post-injection (10 µg/m). (d) Effect of injection of 2.0 female equivalents of OPs on the arylphorin titre at 48 h post-injection (10 µg/m).

The WHv1.0, 1.6 and VHvl.1 genes have been sequenced and are known to be secreted into haemolymph of parasitized animals (Blissard et al., 1989). However, these studies have not elucidated the functional roles of these genes.

A contributing factor to the difficulties encountered in relating polydnavirus genes to specific functional activities is that biochemical assays for the physiological systems disrupted by polydnaviruses have not been developed. In this study the specific effects of CsPDV on an insect storage protein, arylphorin, are described. Arylphorin was selected for study because its expression is both developmentally regulated and associated with insect growth. Arylphorin is a high Mr (approx. 450K) hexameric plasma protein of lepidopteran insects which mature haemolymph protein is a heterohexamer of 74K and 76K glycosylated subunits (LeClerc & Miller, 1990). Synthesis of arylphorin is reduced during intermoult periods as the steroid moulting hormone, 20-hydroxyecdysone, initiates the moulting sequence (Webb & Riddiford, 1988a, b; LeClerc & Miller, 1990). Expression of arylphorin is renewed at the beginning of the fourth and fifth instar, after the conclusion of moulting.

Manduca sexta haemolymph arylphorin is dramatically reduced after parasitization by the braconid wasp Cotesia congregata (Beckage et al., 1989; Beckage & Kanost, 1993). We have observed that H. virescens larvae parasitized by C. sonorensis also have reduced haemolymph arylphorin titres relative to controls. In this study the mechanisms by which CsPDV inhibits arylphorin accumulation in the haemolymph were investigated in order to develop a biochemical assay for the selection and functional analysis of polydnavirus genes affecting arylphorin titres and, ultimately, insect growth.

Methods

Chemicals. Chemicals were purchased from Sigma or Fisher BioTech. All enzymes were purchased from Promega. The radioisotopes [32P]dATP (800 Ci/mmol) and [35S]methionine (1203 Ci/mmol) were purchased from NEN-Dupont.

Animals. Premoult penultimate instar H. virescens larvae were parasitized by C. sonorensis according to the method of Dover et al. (1988). Animals that had not completed the moult to the fifth instar within 12 h after treatment were not used. After 12 or more hours had elapsed, haemolymph was collected from individual larvae onto Parafilm, pipetted directly into sample buffer and then analysed by SDS-PAGE on 10% polyacrylamide gels (Webb & Riddiford, 1988a).

In vitro tissue incubations. Fat bodies were dissected from control and parasitized premoult fourth instar H. virescens larvae and incubated in 100 µl of oxygenated methionine-free Grace’s medium containing 175 µCi [35S]methionine for 6 h in vitro according to Webb & Riddiford (1988a). Grace’s medium and fat body tissue were harvested separately and analysed by SDS-PAGE on 10% polyacrylamide gels. Aliquots of Grace’s medium and fat body homogenate corresponding to 5 x 10^5 c.p.m. were immunoprecipitated using 2 µl of rabbit antiserum against the H. virescens arylphorin large subunit, p76 (LeClerc & Miller, 1990).

Polydnavirus preparation and injections. Calyx fluid was prepared from ovarian calyces freshly dissected from C. sonorensis females by the method of Dover et al. (1988). CsPDV and ovarian proteins (OPs) were purified from calyx fluid by sucrose gradient centrifugation as described by Krell et al. (1982). Premoult penultimate instar H. virescens larvae

storage organs such as the fat body and other tissues. The granules are broken down and the amino acids utilized during subsequent pupal and adult development (Kinnear & Thomason, 1975). Synthesis of arylphorin subunits ceases during the pharate pupal stage and the mRNA for this protein can no longer be detected after wandering begins (LeClerc & Miller, 1990). The 76K subunit of H. virescens arylphorin is translated from a 2.7 kb transcript by the larval fat body and testis. The mature haemolymph protein is a heterohexamer of 74K and 76K glycosylated subunits (LeClerc & Miller, 1990). Synthesis of arylphorin is reduced during intermoult periods as the steroid moulting hormone, 20-hydroxyecdysone, initiates the moulting sequence (Webb & Riddiford, 1988a, b; LeClerc & Miller, 1990). Expression of arylphorin is renewed at the beginning of the fourth and fifth instar, after the conclusion of moulting.

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were injected behind the head capsule. Controls received an equal volume of Pringle's saline. Animals that bled profusely from the injection site were discarded. In preliminary dosage experiments, premoult penultimate instar *H. virescens* larvae were injected with a range from 2.0 to 0.01 female wasp equivalents of calyx fluid, CsPDV or of OPs in a 2 μl volume. Inhibition of growth in 0.01 female equivalent calyx fluid- and 2.0 to 0.1 female equivalents CsPDV-injected larvae was evident by 48 h post-injection. The lower dose of 0.1 female equivalents of CsPDV was adopted for all subsequent experiments. Successful viral infection in parasitized, CsPDV- and calyx fluid-injected animals was verified by Western blotting of haemolymph at 48 h post-treatment using antisera prepared against the CsPDV-encoded VHv1.1 protein (X. Li & B. A. Webb, unpublished results). The presence of OPs in injected animals was confirmed by Western blotting of haemolymph proteins at 48 h post-injection as described by Webb & Summers (1990).

**RNA analysis.** Total RNA was extracted from control and parasitized third, fourth and fifth instar larvae by the method of Webb & Riddiford (1988b). Five μg of total RNA was subjected to in vitro transcription using a Gibco-BRL micrococcal nuclease-treated rabbit reticulocyte lysate kit according to the manufacturer's instructions. Aliquots of 5 x 10^6 c.p.m. were immunoprecipitated and analysed by SDS–PAGE (Webb & Riddiford, 1988b). Northern blots were prepared by electrophoresing 2 to 10 μg of total RNA on 1.2% agarose, 2.2 M-formaldehyde gels. Separated RNA was transferred to nylon membranes and u.v. cross-linked using a Stratalinker (Stratagene). Blots were probed with a [³²P]dATP-labelled 761 bp EcoRI fragment of the p76 gene, at 42 °C, in hybridization solution (50% formamide, 5 x Denhardt's solution, 6 x SSC, 0.5% SDS). Blots were washed twice with 1 x SSC, 0.1% SDS at 37 °C, then twice with 0.2 x SSC, 0.1% SDS at 68 °C and then autoradiographed for 1 to 2 days at ~70 °C. Transcription from the CsPDV genome in insect tissues was monitored with cDNA probes of the VHv1.1 gene (Dib-Hajij et al., 1993). A 1.8 kb PstI fragment of Bombyx mori β-actin was used to ensure equal loading of lanes. A Molecular Dynamics Imagequant 400E Phosphor Imager was used to quantify the level of arylphorin mRNA on selected Northern blots.

**Results**

*H. virescens* larvae parasitized just before molting to the final larval instar exhibited reduced titres of haemolymph arylphorin at 24 and 48 h post-injection relative to unparasitized larvae. Representative haemolymph protein profiles are shown in Fig. 1 (a) (p74/76). Synthesis of the 82K subunits of the other major haemolymph storage protein, riboflavin-binding hexamer, is also inhibited (Fig. 1 a; p82). Although the titre of arylphorin is dramatically affected, the levels of other major proteins such as apolipophorin-I (220K) and insecticyanin-like protein (160K) appear unchanged in parasitized larvae. Unparasitized larvae moult to the ultimate instar, begin feeding, growing and resume synthesis of arylphorin for secretion into the haemolymph. Because storage proteins constitute a significant fraction of total haemolymph protein, their absence leads to a significant decline in plasma protein concentration. At 24 h post-parasitization (p.p.) unparasitized larval plasma protein concentrations were 106 ± 13 mg/ml, whereas parasitized larvae had only 54 ± 27 mg/ml (n = 12). By 48 h p.p. the plasma protein level of unparasitized larvae rose to 298 ± 80 mg/ml, whereas that of parasitized larvae declined to 23 ± 10 mg/ml (n = 10). It should be noted that wasp larvae begin to hatch at approximately 48 h p.p. and therefore would not directly affect arylphorin titre by feeding on haemolymph until later time points.

Many of the physiological effects of parasitization on *H. virescens* growth and development have long been associated with polydnaviruses. To determine whether polydnavirus infection affected arylphorin levels, calyx fluid (containing virus), purified virus or non-viral ovarian proteins were injected into larvae. Concentrations of virus exceeding 0.1 female equivalents and of calyx fluid greater than 0.01 female equivalents inhibited larval growth (data not shown). When 0.01 female equivalents of calyx fluid were injected into larvae, haemolymph arylphorin levels were reduced 48 h later (Fig 1 b). Injection of 0.1 female equivalents of purified CsPDV into larvae also reduced the haemolymph arylphorin titre 48 h post-injection (Fig. 1 c). Finally, injection of up to 2.0 female equivalents of OPs was detected in the haemolymph by Western blotting at 48 h post-injection (data not shown), but no effect on haemolymph arylphorin titre was evident (Fig. 1 d). Because there were no detectable differences between parasitization or injection of virus or calyx fluid on arylphorin titres we utilized either parasitized or calyx fluid-injected larvae for most experiments. The effects of parasitization or calyx fluid injection on haemolymph arylphorin titre can be directly attributed to the polydnavirus.

The decline in haemolymph storage protein titre in parasitized larvae resulted from reduced fat body synthesis of the p76 and p74 arylphorin subunits and the p82 subunits of the riboflavin-binding hexamer (Fig. 2 a). When explanted fat bodies from unparasitized and parasitized larvae 24 and 48 h p.p. were labelled in vitro with [³⁵S]methionine, a marked reduction in the synthesis of p76 arylphorin titre 48 h post-injection (Fig. 1 c). Finally, injection of up to 2.0 female equivalents of OPs was detected in the haemolymph by Western blotting at 48 h post-injection (data not shown), but no effect on haemolymph arylphorin titre was evident (Fig. 1 d). To confirm that the 74K/76K proteins inhibited by parasitization were the arylphorin subunits, a specific rabbit polyclonal antiserum raised against *H. virescens* p76 arylphorin subunit was used to immunoprecipitate [³⁵S]methionine-labelled protein from fat body homogenates. Immunoprecipitates were analysed by SDS–PAGE followed by autoradiography. Parasitization resulted in a significant decline in the in vitro synthesis of immunoprecipitable [³⁵S]labelled p76 arylphorin subunit by fat bodies in two-thirds of larvae by 24 h p.p. and in all larvae by 48 h p.p. (Fig. 2 b). Injection of 0.1 female equivalents of sucrose gradient-purified CsPDV into premoult penultimate instar larvae resulted in a markedly reduced synthesis of p76 by fat bodies when these were explanted and incubated in vitro 48 h
post-injection (Fig. 2c). On the basis of haemolymph protein and fat body labelling experiments, synthesis of the p74 subunit and p82 were similarly inhibited by parasitization, calyx fluid or virus injection.

Many viruses are known to inhibit host transcription. To determine whether steady-state arylphorin mRNA levels were affected by parasitization, total RNA was extracted from control and parasitized larvae and analysed by Northern blotting. Unexpectedly, the results indicated that the arylphorin transcript level was not affected by parasitization (Fig. 3 a). When equal amounts of total RNA from controls and parasitized larvae at 24 and 48 h p.p. were electrophoresed a similar signal was detected. β-Actin levels were monitored by hybridization as an internal standard to ensure equal loading of lanes (Fig. 3 b). Polydnavirus transcription was monitored in these experiments by Northern blotting of total RNA from naturally parasitized and calyx fluid-injected animals. Transcripts from the VHvl.1 gene were evident in parasitized, calyx fluid- and CsPDV-injected larvae at all time points beyond 24 h (data not shown). Additional faint bands were occasionally observed on Northern blots after long exposure. Faint bands of higher $M_e$ were likely to be unspliced arylphorin hnRNA (Webb & Riddiford, 1988b).

The arylphorin transcript level is greatly reduced during intermoult periods. At the conclusion of moulting, transcription and translation of arylphorin resumes. The effect of parasitization on this increase in arylphorin mRNA was examined at 12 h intervals after parasitization (Fig. 3 c). Larvae were parasitized just prior to moulting to the penultimate instar. These intermoult larvae expressed low levels of arylphorin mRNA (Fig. 3 c, lane 1), compared to unparasitized larvae 12 h later (Fig. 3 c, lane 2) and to parasitized larvae at 12 to 72 h p.p. (Fig. 3 c, lanes 3 to 8) or unparasitized controls 48 to 96 h later (Fig. 3 c, lanes 9 to 12). Thus parasitization does not interfere with the normal post-moulting resumption of arylphorin transcription in fat bodies, but translation of this message is blocked.

Reduced larval growth was clearly evident by 48 h p.p. Injection of 0.01 female equivalents of calyx fluid, containing CsPDV, elicits the same physiological symptoms of reduced growth and reduced haemolymph arylphorin titre. To demonstrate that the effects of
parasitization or calyx fluid injection, but protein synthesis could be blocked by rendering the mRNA untranslatable. To address this possibility, mRNA from parasitized larvae was translated in vitro using rabbit reticulocyte lysates (Fig. 4a) and
35S-labelled arylphorin was immunoprecipitated using specific polyclonal antibodies (Fig. 4b). Equivalent levels of a [35S]methionine-labelled 74K protein were synthesized in lysates containing total RNA from either parasitized or unparasitized control larvae. Rabbit antisera prepared against the purified p76 subunit from H. virescens haemolymph immunoprecipitated a 74K protein from reticulocyte lysates, demonstrating that unglycosylated arylphorin is synthesized in vitro. Equal levels of the p76 immunoprecipitable translation product were present in in vitro translations from calyx fluid-injected and control larvae (Fig. 4c). Therefore, arylphorin transcripts are intact and translatable in vitro.

Parasitized larvae exhibited reduced feeding and weight gain compared to unparasitized controls. To determine whether the reduction in arylphorin synthesis was related to nutritional deprivation resulting from reduced feeding, starvation experiments were performed. Experimental larvae were transferred from insect diet to non-nutrient agar blocks for 24 or 48 h. Fasting reduced the arylphorin mRNA detected on Northern blots within 24 h of transfer. By 48 h of starvation arylphorin messenger was undetectable by Northern blotting. In vitro translation of total mRNA from animals fasted for 48 h resulted in substantially less incorporation of isotope into proteins (data not shown).

**Discussion**

We have demonstrated that although the haemolymph titres of arylphorin decline in parasitized animals and the synthesis of arylphorin by the fat body was inhibited, mRNA transcript levels of arylphorin were not affected. Because arylphorin mRNA is present in very low amounts at the time of parasitization, the mRNA must be synthesized after parasitization, but translation is somehow blocked. In vitro translation of RNA from parasitized larvae showed that the arylphorin messenger was undegraded and abundantly translatable in a cap-dependent translation system. Injection of calyx fluid into larvae caused the same symptoms as parasitization: reduced growth, reduced arylphorin titre and synthesis, all without affecting arylphorin mRNA levels. Finally, injection of purified virus, but not of OP, was sufficient to produce these symptoms, including the inhibitory effect on arylphorin translation.

Although translational control during development and viral disruption of host translation during infection are well known (Hershey, 1991; Kozak, 1992), examples...
of such highly specific translational regulation of host transcripts by a virus, as described in this report, have not been reported. Infection with many viruses, such as picornaviruses, adenoviruses and poxviruses, results in a generalized blockade of translation of host mRNA, caused either directly by viral products, or indirectly by cellular defensive reactions (Moss, 1990). For example, poliovirus encodes a protease which cleaves the p220 subunit of eukaryotic initiation factor (eIF) 4F, resulting in a block of host cap-dependent translation (Hershey, 1991). Viruses that disable host translation, or evade the host defensive mechanisms, encode transcripts which can circumvent translational inhibition. Adenovirus infection causes a reduced level of eIF-4E phosphorylation which reduces the activity of the eIF-4F complex on host transcripts (Huang & Schneider, 1991). Translation from host transcripts is rapidly inhibited in influenza virus-infected cells. However, unlike the viruses mentioned above, influenza virus transcripts require cap-dependent initiation (Garfinkel & Katze, 1992), but possess 5'-untranslated regions which confer the ability to circumvent cellular translational inhibition (Garfinkel & Katze, 1993). Alphavirus infection of vertebrate cells results in reduced translation from host mRNA by raising the intracellular cytoplasmic sodium concentration and lowering the potassium concentration. Viral transcripts are translated more efficiently under low potassium conditions (Gary et al., 1979). The baculovirus Autographa californica nuclear polyhedrosis virus inhibits host transcription during early to middle time points post-infection (Ooi & Miller, 1988). A precedent exists for the translational regulation of insect storage protein synthesis during metamorphosis (Sekeris & Scheller, 1977). Synthesis of calliphorin, a protein homologous to arylphorin, by the fat body of the blowfly Calliphora vicina is subject to translational inhibition in the final instar larvae. Translatable mRNA is present at high levels for 3 days after synthesis of the protein cannot be detected in vivo or in vitro (Sekeris & Scheller, 1977). Because of the unique obligate symbiosis which has evolved between the wasp and the polydnavirus (Stoltz & Whitfield, 1992), the mechanism of translational inhibition used by the polydnavirus may be novel.

M. sexta haemolymph arylphorin levels are dramatically reduced after parasitization by the braconid wasp C. congregata (Beckage et al., 1989; Beckage & Kanost, 1993). Moreover, the effect of parasitization on arylphorin and other haemolymph proteins reflects a specific inhibition, limited to several plasma proteins rather than a general decline. For example the titre or activity of the haemolymph proteins arylphorin, juvenile hormone esterase, 20-hydroxyecdysone monoxygenase, prophenoloxidase, apolipophorin-III and serpin levels all decline, whereas lipophorin, haemocyte aggregation-inhibiting protein and insecticyanin levels remain unchanged (Beckage & Kanost, 1993). Trichoplusia ni parasitized by the braconid wasp Chelonus curvimaculatus undergo precocious metamorphosis, exhibiting an increased arylphorin titre (Kunkel et al., 1990). Conversely, a juvenilizing effect, or arrested development, with declining arylphorin titres ensues after parasitization of M. sexta by C. congregata (Beckage et al., 1989), and after parasitization of H. virescens by the ichneumonid C. sonorensis or by the braconid Microplitis croceipes (F. Hossein & D. Dahlman, personal communication).

Why should CsPDV evolve genes which reduce arylphorin synthesis? One simple hypothesis is that translational inhibition of storage protein synthesis is an effect incidental to some other viral function, such as immunosuppression, or disruption of the hormonal milieu. Storage protein messengers might have regulatory sequences in common with the intended target messengers, or possess secondary structures that resemble targets. A second hypothesis posits that wasp larvae benefit from diversion of substantial amounts of amino acids from protein synthesis into the increased plasma amino acid levels seen in parasitized hosts. Host weight gain is substantially inhibited after parasitization. Diversion of host resources away from adult activities such as pupal development and reproduction would clearly benefit both the wasp larva and its viral symbiote.

The reduced feeding and growth exhibited by parasitized H. virescens may have contributed to reduced protein synthesis by host tissues. Parasitization by ichneumonids has been reported to cause fundamental shifts in carbohydrate and amino acid metabolism (Thompson, 1993). Vinson (1990) has shown that parasitization of H. virescens by C. sonorensis results in an increased concentration of trehalose and total amino acids in the host haemolymph. In an attempt to mimic the effect of reduced nutrient uptake, unparasitized larvae were starved for up to 48 h. Fasted insects synthesized less arylphorin, but all protein synthesis was equally inhibited by this treatment. Arylphorin mRNA on Northern blots was greatly decreased by 24 h and completely absent in insects fasted for 48 h. The synthesis of arylphorin and total protein synthesis by the fat bodies of M. sexta was inhibited by fasting (Webb & Riddiford, 1988a). Thus, the reduced feeding caused by parasitization cannot account for the specific inhibition of storage protein synthesis.

It is possible that the apparent inhibition of arylphorin synthesis reflects no change in synthesis but an increased rate of protein degradation, in either the haemolymph or the fat bodies of parasitized larvae. However, no evidence of specific proteolytic activity against arylphorin was observed in labelled immunoprecipitates from fat body
homogenates, or from haemolymph. By contrast, when arylphorin was utilized during *M. sexta* larval molts, immunoreactive proteolytic fragments were readily detected (Webb & Riddiford, 1988a). Because the translational inhibition of arylphorin synthesis can be duplicated by injecting purified virus into larvae, the inhibitory effect is likely to be mediated by a viral gene product. However, when mRNA extracted from parasitized or CsPDV-injected larvae (which include viral transcripts) was translated in *vitro* using rabbit reticulocyte lysates, the translation of endogenous arylphorin messenger was not inhibited. CsPDV proteins may not have accumulated to a level sufficient to inhibit arylphorin translation, or post-translational modifications may be necessary for function. Alternatively, the newly translated CsPDV inhibitory factor(s) were not active in the heterologous rabbit reticulocyte system.

We have shown that injection of CsPDV is necessary and sufficient to cause the translational inhibition of arylphorin synthesis in the fat bodies of *H. virescens* larvae. Arylphorin mRNA levels are unaffected by this treatment but translation appears to be inhibited in infected larvae by an as yet unidentified polydnavirus gene product. We are currently screening several candidate CsPDV genes for their effect on arylphorin translation.

We thank Steve Miller for providing the pHV-2 clone of arylphorin and the p76 antisera used in these experiments and Nicole Mounier for providing the *Bombyx mori* γ-actin probe. Also we would like to thank Peter Derks for assistance with the phosphor imager. This work was supported by a grant from American Cyanamid, Inc., and project 08180 of the New Jersey Agricultural Experimentation Station. New Jersey Agricultural Experiment Station publication #D-08180-01-94 supported by state funds and by U.S. Hatch Act.

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(Received 1 February 1994; Accepted 15 April 1994)