Persistence and expression of *Microplitis demolitor* polydnavirus in *Pseudoplusia includens*


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Persistence and expression of *Microplitis demolitor* polydnavirus (MdPDV) was examined in parasitized and virus-injected *Pseudoplusia includens* larvae. Viral DNA persisted in *P. includens* larvae for 6 days, but no increase in the amount of viral DNA present was detected. Viral transcripts were observed in parasitized and virus-injected larvae 4 h post-parasitism and expression continued for 6 days. When specific host tissues were examined, more viral DNA and RNA was detected in haemocytes than in the gut, nervous system and fat body. \(^{32}\)P-labelled MdPDV DNA hybridized to approximately six different size classes of mRNAs on Northern blots of RNA from haemocytes of parasitized larvae. MdPDV transcription was first detected in haemocytes at 4 h post-parasitism and continued for 6 days. Similar transcripts were observed in haemocytes from larvae that had been injected with calyx fluid or MdPDV plus venom. First-strand cDNA probes of haemocyte-specific MdPDV transcripts hybridized to only certain MdPDV viral DNAs, suggesting that only part of the MdPDV genome is expressed in this host cell type.

Introduction

Critical to the development of many endoparasitic wasps (parasitoids) is the ability to avoid elimination by the immune system of their host. Parasitoids are often killed in non-permissive hosts via a cell-mediated encapsulation response in which host blood cells (haemocytes) form a capsule around the parasitoid (Salt, 1968; Lackie, 1988). In permissive hosts, however, parasitoids avoid encapsulation by either possessing surface features that passively protect the parasitoid from encapsulation (Salt, 1968; Davies & Vinson, 1986; Feddersen et al., 1986) or suppressing the encapsulation response directly via factors the female wasp injects into the host at oviposition (Edson et al., 1981; Rizki & Rizki, 1984; Guzo & Stoltz, 1987; Davies et al., 1987; Tanaka, 1987; Strand & Noda, 1991).

Of particular interest in the suppression of the host encapsulation response is the role of viruses in the family Polydnaviridae. Polydnaviruses are found in wasps in the families Braconidae and Ichneumonidae, and are characterized by double-stranded, superhelical DNA genomes that are heterologous in size (Stoltz et al., 1984). The polydnaviruses carried by braconid and ichneumonid wasps differ morphologically and are currently recognized as two genera. The bracoviruses have cylindrical nucleocapsids surrounded by a single unit membrane envelope whereas the ichnoviruses have fusiform nucleocapsids and are surrounded by two unit membranes. Recent studies suggest that the DNA of polydnaviruses is integrated into the genome of both male and female wasps, and that transmission is vertical (Fleming & Summers, 1986, 1991; Stoltz, 1990; Xu & Stoltz, 1991). Viral replication occurs only in the calyx region of the female wasp with the resulting suspension of polydnavirus and protein being referred to as calyx fluid (Stoltz & Vinson, 1979). When the wasp oviposits, one or more eggs and a quantity of calyx fluid are injected into the wasp's host. Studies with the ichnovirus of *Campoletis sonorensis* and bracovirus of *Cotesia melanospicula* indicate that viral transcription in the apparent absence of replication occurs in the host larva within hours of wasp oviposition (Fleming et al., 1983; Theilmann & Summers, 1986; Stoltz et al., 1988; Blissard et al., 1989; Webb & Summers, 1990). It is unknown, however, whether viral expression occurs in all or only certain host tissues.

Suppression of the encapsulation response in *Pseudoplusia includens* (Lepidoptera: Noctuidae) after parasitism by the braconid wasp *Microplitis demolitor* is due to alterations in the spreading of haemocytes (plasmatocytes and granular cells) that participate in capsule formation (Strand & Wong, 1991; Strand & Noda, 1991). Injection of physiological doses of *M. demolitor* calyx fluid or purified bracovirus (MdPDV) causes similar alterations, but the effects of MdPDV are abolished if...
the viral genome is cross-linked in situ with psoralen before injection into the host. This would suggest that one or more MdPDV gene products is responsible for immunosuppression. In the present study we examined the persistence and expression of MdPDV in *P. includens*. Here we report that MdPDV persists for 6 days in hosts without apparent replication. MdPDV expression was detectable in *P. includens* 4 h after oviposition by *M. demolitor* and continued for 6 days. Examination of specific host tissues indicated that the highest levels of viral expression were associated with host haemocytes.

**Methods**

*Insects.* *P. includens* larvae were reared and staged as described previously in 30 ml plastic cups with paper lids at 27 ± 1 °C and a 16 h light (L): 8 h dark (D) photoperiod (Strand, 1990). Moths were fed a 20% sucrose solution. *M. demolitor* were reared at 27 ± 1 °C and a 16L: 8D photoperiod as outlined by Strand & Wong (1991). *P. includens* were 6- to 12-h-old fourth stadium larvae when parasitized or injected with wasp components. Unparasitized larvae served as negative controls and 2-day-old adult wasps served as positive controls.

**Calyx fluid collection and virus purification.** The reproductive tracts of female wasps were excised under saline by gently pulling the ovipositor from the abdomen. Venom glands and calyces were removed and placed either separately or together into drops of saline. The calyces and reservoirs of the venom glands were then punctured using forceps and the contents allowed to diffuse into the drop. Calyx and venom were then diluted in Pringle's saline (Pringle, 1938) to the appropriate concentration for use in injection experiments. Quantities of calyx fluid and venom injected were based on wasp equivalents. MdPDV was purified on sucrose gradients as described by Strand & Noda (1991). Briefly, calyx fluid from 50 to 100 wasps was collected and filtered through a 0.45 μm cellulose acetate syringe filter to remove the parasite eggs and other debris. The calyx fluid was then loaded onto a 25 to 50% sucrose gradient (made in Pringle's saline) and centrifuged at 42000 g for 20 min in a Beckman TLS-55 rotor using a Beckman TL-100 ultracentrifuge. The virus was diluted in 2 ml of Pringle's saline and repelleted by centrifugation at 25000 g for 1 h. For use in injection experiments, MdPDV was resuspended to the appropriate volume in saline.

**Electron microscopy.** The reproductive tracts from adult females were dissected as described previously and fixed in 3% glutaraldehyde in phosphate buffer pH 7.4 at 4 °C. After rinsing in buffer, tissues were post-fixed in 2% osmium tetroxide, rinsed in distilled water and block-stained in 1% uranyl acetate prior to being embedded. Tissues were dehydrated in graded ethanol solutions, transferred to 100% acetone and embedded in Epon. Tissues were sectioned on a Sorvall MT-2B ultramicrotome and sections were stained in uranyl acetate and lead citrate before examination by transmission electron microscopy (Strand et al., 1986). To observe MdPDV after sucrose gradient purification, particles were examined by scanning electron microscopy. Gradient-purified MdPDV in Pringle's saline was placed directly on acid-cleaned, poly-L-lysine-coated coverslips and fixed in 3% glutaraldehyde as described previously. After post-fixation in 1% osmium tetroxide, particles were dehydrated in graded ethanol solutions, critical point-dried and coated with gold.

**Isolation and labelling of viral DNA.** MdPDV from 50 wasps was diluted to 100 μl in saline and digested for 2 h at 37 °C with 2-6 μl proteinase K (10 mg/ml), 1 μl of 0.5 mM-EDTA, and 10 μl KCl (1 M). Sarkosyl was added to a final concentration of 4% and the sample was incubated for 15 min at 68 °C. MdPDV DNA was then extracted with equal volumes of phenol:chloroform, and ethanol-precipitated in the presence of 0.30 M-sodium acetate pH 5.2. MdPDV DNA was labelled by nick-translation with [32P]dCTP (400 Ci/mmoll) (Amersham) to high specific activity (1 × 10⁵ to 2 × 10⁶ c.p.m./μg) (Rigby et al., 1977).

**Isolation of DNAs and RNAs from P. includens.** For specific experiments, *P. includens* larvae were either parasitized individually by *M. demolitor*, or injected with 0.02 wasp equivalents of calyx fluid or 0.2 equivalents of purified MdPDV plus venom (Strand & Noda, 1991). An increased amount of purified MdPDV was injected relative to calyx fluid to compensate for the potential loss of viable virus during purification. Although venom itself does not affect host development or haemocyte behaviour, its presence appears to synergize the effects of MdPDV (Strand & Noda, 1991; Strand & Dover, 1991). Similar synergistic effects of venom have been observed with other polynuclear-virus-carrying bracoidis (Kitano, 1982; Stoltz et al., 1988). For injection experiments, larvae were anaesthetized with CO2 and injected through a probe using a glass needle mounted on a micromanipulator.

**For analysis of MdPDV persistence and expression in *P. includens*, cohorts of six larvae were selected at designated times post-parasitism or post-injection and homogenized in an equal volume of phenol:chloroform and lysis buffer (0.5% SDS, 0.20 M-NaCl, 25 mM-EDTA, pH 8.0). For analysis of MdPDV in specific host tissues, groups of parasitized or calyx fluid-plus-venom-injected larvae were dissected in Pringle's saline, and the gut (digestive tract and Malpighian tubules), nervous system (brain and ventral nerve chord) and fat body were collected. It should be noted that some tracheal tissue was associated with the gut and fat body. Haemocytes were collected by bleeding larvae from a cut proleg into saline and pelleting the cells at 400 g. Tissues or haemocytes were then placed immediately in phenol:chloroform and lysis buffer (1:1). DNA and RNA were extracted from tissues as outlined by Sambrook et al. (1989). Integrity of the RNA samples was confirmed by visualization of ribosomal RNA on 1-2% agarose-formaldehyde gels. To monitor the efficiency of DNA recovery during characterization of MdPDV persistence in parasitized hosts, pGEMBX (a plasmid construct containing bovine herpesvirus gB promoter; D. 1. McKenzie, unpublished) DNA was added to samples as an internal standard. Purified DNA was stored in H2O at 4 °C and RNA was stored in H2O at -80 °C.

**cDNA synthesis.** First-strand cDNA probes were synthesized from RNA isolated from *P. includens* haemocytes. Approximately 10 μg of total RNA was reverse-transcribed in the presence of 0.5 mM- dATP, -dGTP, -dTTP and 100 μCi [α-32P]dCTP (Amersham, 3000 Ci/mmol), 1 μg oligo(dT)12-18 (Pharmacia) and 200 units of Moloney murine leukaemia virus reverse transcriptase for 1 h at 37 °C. The reaction was then spiked with 0.5 mM-ATP (final concentration) and another 100 units reverse transcriptase. Free [32P]dCTP was removed from the sample by selective precipitation of the cDNA in ethanol and 2 M-ammonium acetate. The final pellet was washed with cold 70% ethanol and suspended in 100 μl of water.

**Gel electrophoresis and blot hybridizations.** For slot blot experiments, DNA from whole *P. includens* larvae or particular tissues was digested with HindIII, transferred to nitrocellulose using a slot blot manifold (Bethesda Research Laboratories) and hybridized to 32P-labelled MdPDV DNA. Total RNA from *P. includens* larvae or particular tissues was similarly transferred to nitrocellulose and probed with 32P-labelled MdPDV DNA. Hybridizations were performed as outlined by Kafatos et al. (1979). To estimate the amount of MdPDV DNA present per parasitized larva, the amount of radioactive label hybridized to each slot was determined using a radioanalytic imaging system (AMBIS Systems).
For Northern blots, total RNA from *P. includens* haemocytes was size-fractionated on 6% formaldehyde, 10% agarose gels and transferred to nitrocellulose in 10× SSC (Sambrook *et al.*, 1989). Hybridization with 32P-labelled MdPDV DNA was carried out at 42°C in 5× SSC, 0.1% SDS, 10× Denhardt’s solution and 50% formamide. Northern blots were washed in 1× SSC, 0.1% SDS at 68°C. For Southern blot hybridizations of first-strand cDNAs from *P. includens* haemocytes to MdPDV DNA, undigested or HindIII-digested MdPDV DNAs were size-fractionated on 1% agarose gels and transferred to nitrocellulose. Hybridization conditions were the same as described for the Northern blots.

**Results**

**Virus purification**

Ultrastructural examination indicated *M. demolitor* calyx fluid contained a homogeneous mixture of elongate virions (Fig. 1a). Each virion consisted of one cylindrical nucleocapsid (35 nm wide and 35 to 50 nm long) enveloped by a single unit membrane (Fig. 1b to d). Although the envelope of MdPDV varied in length, a distinct protrusion was consistently visible at one end of the membrane. Nucleocapsid length was only slightly variable. Centrifugation of filtered calyx fluid on sucrose gradients concentrated the virus particles into a thin light blue band. The virus was of homogeneous morphology and free of contamination when viewed by scanning electron microscopy (Fig. 1d).

Based on the amount of viral DNA recovered from six replicate samples of 50 *M. demolitor* calyces, individual female wasps were estimated to contain 154.5 ± 21.3 ng of viral DNA. Extracted MdPDV DNAs fractionated into at least 15 bands on 1% agarose gels (Fig. 2). The fluorescence of some of the DNA bands (e.g. A, G, H and M) was higher than others, and could represent multiple DNAs of similar size. However, the relative fluorescence of the different bands was constant over numerous collections of the virus from *M. demolitor* females. MdPDV DNAs digested with EcoRI or HindIII fractionated into approximately 28 and 30 bands respectively (Fig. 2). Assuming each restriction fragment is unique, the aggregate size of the MdPDV genome was estimated to be 164.5 kb for EcoRI fragments and 169.2 kb for HindIII fragments.

**MdPDV persistence and expression in *P. includens***

To determine whether MdPDV persists in parasitized and calyx fluid-injected larvae, DNA was isolated from host larvae at sequential periods and hybridized with 32P-labelled MdPDV DNA. Slot blot analysis indicated that viral DNA persisted in the host through the course of parasitism at approximately the same level as that present immediately after oviposition by the wasp (Fig. 3a). In parasitized hosts the *M. demolitor* egg hatches 26 to 28 h post-parasitism and the final instar larva emerges from the host at 156 to 162 h (days 6 to 7) to pupate (Strand & Wong, 1991). Thus, each parasitized *P. includens* larva contained a wasp in the egg or larval stage. Although no parasitoid was present in hosts injected with calyx fluid, the same pattern of viral persistence was observed. 32P-labelled MdPDV DNA did not hybridize to DNA from unparasitized *P. includens* but did hybridize to DNA from *M. demolitor* female wasps (Fig. 3a). In addition, no hybridization was detected between 32P-labelled MdPDV DNA and DNA from hosts injected with only *M. demolitor* venom (data not presented). Quantification of DNA blots from parasitized hosts by radioanalytic image analysis indicated that the amount of MdPDV DNA present per host ranged from 0.8 to 3.6 ng over the 6 day period of parasitism (Fig. 3b).

To determine whether MdPDV expression occurs in parasitized and calyx fluid-injected larvae, RNA was isolated from hosts at various times and probed with 32P-labelled MdPDV DNA. Slot blot analysis indicated no hybridization was detectable at 2 h post-parasitism, but a hybridization signal was present at 4 h and remained detectable for 6 days (Fig. 4). MdPDV transcripts were also detected from 4 h to 6 days in *P. includens* larvae injected with calyx fluid (Fig. 4). No hybridization was detected between 32P-labelled MdPDV DNA and RNA from unparasitized *P. includens*.

**Expression of MdPDV in tissues of *P. includens***

Examination of *P. includens* larvae at 24 h post-parasitism indicated that the presence and expression of MdPDV varied with host tissue. Careful collection of specific tissues or haemocytes assured that no *M. demolitor* egg or larva was present in the samples. Hybridization between 32P-labelled MdPDV DNA and DNA from host haemocytes and nervous tissue was detectable by slot blot analysis after a 6 day exposure (Fig. 4). After a 14 day exposure, however, hybridization between MdPDV DNA and DNA from host fat body and gut was also detected (data not shown). The highest level of hybridization between 32P-labelled MdPDV DNA and RNA from parasitized hosts was also associated with haemocytes (Fig. 4). Intermediate levels of hybridization were detected in host nervous system tissue, and the lowest levels of hybridization were detected in the fat body and gut. No hybridization was detected in unparasitized hosts or male wasps, but a hybridization signal was detected with DNA and RNA from female wasps (Fig. 4).
Fig. 1. Electron micrographs of MdPDV. (a) Calyx fluid between two parasitoid eggs (E) in the lumen of the lateral oviduct of a 2-day-old *M. demolitor* female. The chorions of the parasitoid eggs are indicated by arrowheads. Bar marker represents 12 μm. (b) Virus particles that form the calyx fluid. Each virion consists of a single cylindrical nucleocapsid (35 nm wide × 35 to 50 nm long) (arrowheads) enclosed by a unit membrane envelope of variable length (200 to 900 nm). Bar marker represents 0.6 μm. (c) Higher magnification of MdPDV nucleocapsids. Bar marker represents 100 nm. (d) Virions after sucrose gradient purification as viewed by scanning electron microscopy. Note the protrusion (arrowheads) of the unit membrane associated with several of the virions. Bar marker represents 300 nm.
Fig. 2. Gel electrophoretic profiles of undigested (lane 1), EcoRI-digested (lane 2) and HindIII-digested (lane 3) MdPDV DNAs. DNAs were loaded at 2 μg per lane and separated in 1% agarose gels. Some of the major undigested bands are indicated by uppercase letters to the right of lane 1 and some of the major bands in the HindIII digests of MdPDV DNA are indicated by lowercase letters to the right of lane 3. Fragments smaller than 2 are indicated by double, lowercase letters beginning with aa. The numbers to the left of lane 1 represent sizes of HindIII-digested λ and PM2 DNA markers. A detailed examination of the genome organization of MdPDV will be presented separately (M. R. Strand, D. I. McKenzie, V. Grassl & J. M. Aiken, unpublished results).

Temporal expression of MdPDV mRNAs in P. includens haemocytes

$^{32}$P-labelled MdPDV DNA hybridized to approximately six different size classes of mRNAs on Northern blots of RNAs from haemocytes of parasitized larvae (Fig. 5). Transcripts were first detected in haemocytes at 4 h post-parasitism and continued for 6 days. $^{32}$P-labelled MdPDV DNA did not hybridize to RNA from haemocytes of unparasitized larvae. Similar size classes of MdPDV mRNAs were produced in haemocytes from hosts that had been injected 24 h previously with calyx fluid or purifiedMdPDV plus venom. mRNAs of 6.8 and 3.6 kb appeared to be the most abundant MdPDV-specific transcripts associated with haemocytes (Fig. 5).

To determine how many MdPDV DNAs were associated with transcription in haemocytes from parasitized P. includens, first-strand cDNA probes were synthesized from haemocyte RNA and hybridized to MdPDV DNA. These probes hybridized with approximately equal intensity to MdPDV DNAs O and K (Fig. 6). Hybridization to HindIII fragments of MdPDV DNA more clearly indicated that only certain MdPDV sequences were represented in the cDNAs from haemocytes of parasitized larvae (Fig. 6). Approximately 30 fragments were observed in ethidium bromide-stained HindIII digests of MdPDV DNA, but only seven
fragments (d, f, g, m, n, y and aa) ranging in size from 12.12 to 1.04 kb hybridized with cDNAs from haemocytes. No hybridization signal was observed between cDNAs synthesized from haemocyte RNA of unparasitized larvae (data not presented).

Discussion

The morphology and genomic organization of MdPDV are consistent with the characteristics of other braconviruses reported in the literature (Stoltz & Vinson, 1979; Stoltz et al., 1984). Although several physiological studies indicate that polydnaviruses are essential for survival of many ichneumonid and braconid wasps (Stoltz & Vinson, 1979; Vinson, 1990), information on viral persistence and expression in parasitized hosts has until now been restricted to C. sonorensis ichnovirus in Heliothis virescens (Fleming et al., 1983; Blissard et al., 1986, 1989; Theilmann & Summers, 1986, 1987) and C. melanoscela bracovirus in Lymantria dispar (Stoltz et al., 1988). Here we report that MdPDV persists and is expressed in P. includens larvae for at least 6 days after parasitism by M. demolitor. Quantitative studies indicated that an average of 2.4 ng of MdPDV DNA is injected into a P. includens larva by M. demolitor at oviposition. Based on the assumption that M. demolitor females contain 150 ng of MdPDV DNA per reproductive tract, a female wasp injects approximately 0.02 wasp equivalents of MdPDV into a host at oviposition. This estimate is consistent with previous dose–response studies that indicated that injection of 0.01 or more wasp equivalents of calyx fluid plus venom alters growth and suppresses the encapsulation response of P. includens in a manner consistent with natural parasitism (Strand & Dover, 1991; Strand & Noda, 1991).

P. includens larvae gain almost no weight after parasitism by M. demolitor (Strand & Dover, 1991). Thus, if MdPDV replicates in P. includens, we would expect to observe an increase in the amount of MdPDV DNA present per host with time. Quantification studies, however, indicated no increase in the amount of viral DNA present per host throughout the 6 day period necessary for the M. demolitor egg and larva to complete development. MdPDV DNA also persisted without apparent increase in hosts injected with calyx fluid plus venom indicating that persistence does not depend on
the presence of the developing parasitoid. Collectively, these data suggest that MdPDV either does not replicate in *P. includens* or replicates at a very low level. In contrast, MdPDV replication is known to occur in *M. demolitor* females during the pupal and adult stage (Strand & Dover, 1991; M. R. Strand, D. I. McKenzie, V. Grassl & J. M. Aiken, unpublished results). In the only other quantitative study on polydnavirus persistence, Theilmann & Summers (1986) also found no evidence for replication of *C. sonorensis* polydnavirus in *H. virescens* although, again, viral replication does occur in female pupae and adults. Studies with *C. sonorensis* and *Hyposoter fugitivus* strongly suggest that ichnovirus DNAs are integrated into the wasp's chromosomal DNA and that transmission is vertical (Fleming & Summers, 1986, 1991; Stoltz *et al.*, 1986; Xu & Stoltz, 1991). Whether bracoviruses are also transmitted vertically is unknown, although genetic studies with *C. melanoscela* are consistent with chromosomal inheritance (Stoltz, 1990). Thus, although polydnaviruses may persist in parasitized hosts, replication of the virus and subsequent ingestion of host tissues by the parasitoid appear unnecessary for transmission.

The entry of polydnaviruses into host tissues has been documented by electron microscopy for several species of wasps (Stoltz & Vinson, 1979), but sites of viral expression in parasitized hosts have not previously been investigated. Tissue-specific differences in expression might, however, be expected since polydnaviruses are known to cause specific alterations in the growth and immune response of hosts after parasitism (Davies *et al.*, 1987; Guzo & Stoltz, 1987; Tanaka *et al.*, 1987; Dover *et al.*, 1987; Strand & Dover, 1991; Strand & Noda, 1991). MdPDV expression in parasitized hosts was first detected 4 h post-parasitism and continued for 6 days at what appeared to be high levels. However, when specific tissues of *P. includens* were examined, more MdPDV DNA was detected in haemocytes than in nervous tissue, fat body or gut. The highest level of MdPDV expression was also associated with host haemocytes, although we emphasize that lower levels of expression were detected in other tissues. It is possible that haemocytes are disproportionately infected by MdPDV because the wasp oviposits into the haemocoel of the host and they are the first cell type encountered by the virus. In contrast, other tissues such as the fat body are ensheathed by a basal lamina and may be less accessible. Because of the morphology of insects, gut and fat body samples also contained trachea that conceivably could contribute to the MdPDV expression signal observed in these tissues. Although polydnaviruses have been observed to enter muscle cells (Stoltz & Vinson, 1979), we did not examine this tissue in the current study because of the difficulty associated with collecting material free of large amounts of trachea and cuticular epithelium.

We should note that MdPDV expression was also detected in adult female wasps but not in males. Male and female wasps were used in this study as a control for monitoring MdPDV expression in *P. includens*. However, these data are consistent with the observation that MdPDV replicates in the ovaries of 2-day-old female wasps, and suggests that the expression we detected is associated with replication. In contrast, our failure to detect MdPDV DNA or RNA in male wasps was probably due to our experimental conditions since only low levels of polydnavirus DNA have been detected in male wasps for other species (Fleming & Summers, 1986).

We detected six MdPDV mRNAs in *P. includens*...
haemocytes beginning at 4 h post-parasitism and continuing for 6 days. Similar transcripts were also observed in hosts injected with calyx fluid or purified virus, again indicating that viral activity is independent of the presence of a developing parasitoid. A 1.7 kb transcript appeared to be the most abundant MdPDV mRNA in haemocytes, and its persistence may indicate a function that is required continuously. A 3.2 kb transcript was also abundant for 24 h but then appeared to decline. At this time, however, we do not know whether these differences represent changes in MdPDV transcription, differential accumulation of stable transcripts or differences in viral expression in particular types of haemocytes. Nevertheless, the pattern of transcription corresponds temporally with the physiological alterations that occur in host plasmatocytes and granular cells after injection of MdPDV (Strand & Wong, 1991; Strand & Noda, 1991). Within 4 h of injection of a physiological dose of MdPDV, both haemocyte types assume abnormal morphologies and lose their ability to encapsulate M. demolitor. All of the morphologically recognizable haemocyte types associated with P. includens remain in circulation during the 6 day period associated with M. demolitor development although the proportion of granular cells declines. Since psoralen cross-linking of MdPDV DNA eliminates its effects on haemocyte spreading and encapsulation in P. includens (Strand & Wong, 1991; Strand & Noda, 1991), the early and continued expression of MdPDV DNA in haemocytes is consistent with the suggestion that disruption of the host encapsulation response is due to viral transcription in blood cells.

Southern blot analysis indicated that haemocyte-specific MdPDV mRNAs hybridize to only a portion of the MdPDV genome. Our recent studies indicate that undigested MdPDV DNAs migrating slower than the 23-5 kb λ HindIII marker may be a mixture of relaxed circular and superhelical DNAs (M. R. Strand et al., unpublished results). Thus, cDNA-positive bands O and K may represent different DNAs or conformational differences in the same DNA. Physical mapping of MdPDV DNAs and examination of their expression in female wasps will be necessary to clarify these relationships. However, this is the first study suggesting polydnavirus expression may vary with tissue in parasitized hosts. Our discovery that expression is associated primarily with host haemocytes is consistent with the immunosuppressive effects of MdPDV in P. includens. Further characterization of MdPDV expression in specific types of haemocytes will undoubtedly improve our understanding of the role of these unusual viruses in parasitism.

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