Transcriptomic profiling of Microplitis demolitor bracovirus reveals host, tissue and stage-specific patterns of activity

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The polydnaviruses (PDVs) are a family of DNA viruses that are symbiotically associated with parasitoid wasps. The transcription of particular genes or gene-family members have been reported for several PDVs, but no studies have characterized the spatio-temporal patterns of expression for the entire complement of predicted genes in the encapsidated genome of any PDV isolate. The braconid wasp Microplitis demolitor carries the PDV Microplitis demolitor bracovirus (MdBV) and parasitizes larval stage Pseudoplasia (Chrysodeixis) includens. The encapsidated genome consists of 15 genomic segments with 51 predicted ORFs encoding proteins ≥ 100 aa. A majority of these ORFs form four multimember gene families (ptp, ank, glc and egf) while the remaining ORFs consist of single copy (orph) genes. Here we used RT-PCR and quantitative real-time PCR methods to profile the encapsidated transcriptome of MdBV in P. includens and M. demolitor. Our results indicate that most predicted genes are expressed in P. includens. Spatial patterns of expression in P. includens differed among genes, but temporal patterns of expression were generally similar, with transcript abundance progressively declining between 24 and 120 h. A subset of ptp, ank and orph genes were also expressed in adult female but not male M. demolitor. Only one encapsidated gene (ank-H4) was expressed in all life stages of M. demolitor, albeit at much lower levels than in P. includens. However, another encapsidated gene (orph-B1) was expressed in adult M. demolitor at similar levels to those detected in P. includens.

INTRODUCTION

Viruses in the family Polydnaviridae consist of two genera, genus Bracovirus (BV) and genus Ichnovirus (IV), that are symbiotically associated with 30–40 000 species of parasitoid wasps (Hymenoptera) in the families Braconidae and Ichneumonidae (summarized by Webb & Strand, 2005; Dupuy et al., 2006; Strand, 2009, 2010). Most polydnavirus (PDV)-carrying wasps parasitize the larval stage of Lepidoptera (moths and butterflies). Each PDV carried by a given wasp species is genetically unique and exists in two forms. In wasps, PDVs persist and are transmitted to offspring as stably integrated proviruses with replication restricted to specialized calyx cells in the ovaries of females. Replication results in production of the encapsidated form of the virus, which accumulates in the lumen of the calyx and is injected into hosts when a wasp oviposits. PDVs do not replicate in the wasp’s host but they do express gene products that cause changes in host immune defences and development that allow the offspring of the wasp to develop successfully.

The encapsidated genomes of PDVs are segmented and consist of multiple circular dsDNAs that are packaged into virions in non-equimolar amounts (Fleming, 1992; Stoltz, 1993; Webb & Strand, 2005; Strand, 2010). These genomic DNAs characteristically exhibit very low coding densities that include a mixture of single-copy genes and multi-member gene families whose known functions involve interactions with the wasp’s host (summarized by Strand, 2010). In contrast, the encapsidated genomes of PDVs lack most (IVs) if not all genes (BV) required for replication, which remain a part of the proviral and/or wasp genome (Bézier et al., 2009; Volkoff et al., 2010). The encapsidated genomes of four BVs [Cotesia congregata bracovirus (CcBV) (Espagne et al., 2004), Microplitis demolitor bracovirus (MdBV) (Webb et al., 2006), Glypta tapanteles indiensis bracovirus (GiBV) and Glypta tapanteles flavicoxis bracovirus (GfBV) (Desjardins et al., 2008)] and four IVs [Campoletis sonorense ichnovirus (CsIV) (Webb et al., 2006), Hyposoter fugitivus ichnovirus (HfIV) (Tanaka et al., 2007), Transcone rostrale ichnovirus (TrIV) (Tanaka et al., 2007) and Glypta funiferae ichnovirus (GfIV) (Lapointe et al., 2007)] have been sequenced while partial sequence data are available for a few other PDV isolates including Chelonus inanitus bracovirus (GdBV) (Weber et al., 2007) and Cotesia plutellae bracovirus (CpBV) (Choi et al., 2009).

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A supplementary table of primers used to amplify predicted ank and single-copy (orph) genes for the RT-PCR and qRT-PCR assays is available with the online version of this paper.
The transcription of particular genes or gene-family members has been reported for several PDVs (summarized by Webb & Strand, 2005; Strand, 2010). However, no studies have characterized the activity of the entire predicted transcriptome from the encapsidated genome of a PDV. The braconid Microplitis demolitor parasitizes larvae of the noctuid moth Pseudoplusia (Chrysodeixis) includens and carries MdBV. The encapsidated genome of MdBV consists of 15 genomic segments, which have an aggregate size of 189 kbp (Webb et al., 2006; Beck et al., 2007). Among these segments, a few predicted ORFs encoding proteins \( \geq 100 \text{ aa} \). Thirty of these ORFs form four gene families: the protein tyrosine phosphatase (ptp) genes (13 members), the ankyrin (ank) genes (12 members), the egf genes (three members) and the glc genes (two members) (Webb et al., 2006). ptp family members share homology with cytosolic ptps from diverse organisms, while all ankyrin family members contain ankyrin repeat domains that are similar to those present in inhibitor \( \kappa B \) (I\( \kappa B \))s proteins. egf family members are named for a shared cysteine-rich domain (epidermal growth factor-like) while glc (glycosylated) family members possess near identical mucin-like domains that are organized in tandem arrays (Strand et al., 1997; Trudeau et al., 2000). In contrast, in most cases the single copy ORFs share no significant homology with known genes in existing databases.

Both microarray and sequencing approaches have been used to identify transcriptional units from insect DNA viruses such as baculoviruses (Yamagishi et al., 2003; Iwanaga et al., 2004; Jiang et al., 2006; Katsuma et al., 2011). However, because of: (i) the relatively small number of predicted ORFs in the encapsidated genome of MdBV, (ii) an absence of overlapping sense and antisense transcriptional units in the MdBV genome (Webb et al., 2006) and (iii) our need for spatio-temporal data spanning multiple tissues and life stages in two insects (\( P. \) includens and \( M. \) demolitor), we used a combination of RT-PCR and quantitative real-time PCR (qRT-PCR) methods to profile the encapsidated transcriptome of MdBV. Our results reveal complex spatial patterns of expression in \( P. \) includens, while also indicating that a subset of MdBV genes is transcribed in \( M. \) demolitor.

### RESULTS

**MdBV genes exhibit complex spatial patterns of expression in \( P. \) includens**

Early Southern and Northern blotting studies using total viral DNA as a probe suggested that MdBV preferentially infects the haemocytes of hosts like \( P. \) includens, and that viral gene expression is detected from 2 h to 6 days post-infection (Strand et al., 1992; Strand, 1994). Subsequent qRT-PCR data elaborated on these results by showing that MdBV genomic segments in host haemocytes are fourfold more abundant than in the host nervous system and fat body, are eightfold more abundant than in the gut and are 40-fold more abundant than in the salivary glands (Beck et al., 2007). However, characterization of the ptp family showed that while transcript abundance is highest in host haemocytes for some family members it is not for others (Pruijssers & Strand, 2007). To expand on these data, we assessed the activity of the predicted transcriptome of MdBV by first conducting RT-PCR studies using primers designed to detect transcripts from most (46 of 51) of the predicted ORFs. We excluded ptp-D1 from the study because prior results showed that it is a pseudogene. We also excluded ptp-H4 because it is truncated but otherwise identical to ptp-J1 (Pruijssers & Strand, 2007). We similarly excluded egf1.5 and glc3.2 because they too are almost identical to egf1.0 and glc1.8, respectively, and cannot be distinguished from one another by PCR-based methods (Webb et al., 2006; Lu et al., 2010). Overall, our results revealed complex patterns, with some predicted ORFs preferentially detected in particular tissues, others detected in most or all host tissues and some not detected in any host tissue (Fig. 1). As controls, we detected no amplicons for any predicted MdBV gene when: (i) template from infected samples was not reverse-transcribed, indicating that our RNA samples were not contaminated with viral DNA, and (ii) reverse transcribed template from non-infected \( P. \) includens was used (data not shown).

Key observations for each gene family and the single-copy genes from the RT-PCR data presented in Fig. 1 included the following. For the ptp family, transcripts were detected for most genes in multiple tissues (Fig. 1a). However, transcripts for ptp-H2, ptp-J1 and ptp-J2 appeared to be preferentially expressed in haemocytes, while transcripts for ptp-H3, ptp-N1 and ptp-N2 appeared to be preferentially expressed in the gut (Fig. 1a). In addition, very weak or no amplicons were observed for ptp family members in host salivary glands (Fig. 1a). Several ank family members were also detected in multiple tissues with amplicons for ank-F5 and ank-H4 being detected in every host tissue we sampled, and ank-I1, ank-N4 and ank-N5 being detected in all tissues with the possible exception of the salivary glands (Fig. 1b). In contrast, ank-G4 appeared to be preferentially expressed in the fat body, while ank-N1 appeared to be preferentially expressed in the gut (Fig. 1b). In addition, very weak or no amplicons were detected in host tissues for ank-C1, ank-C2 and ank-G3 (Fig. 1b). egf1.0/egf1.5 expression was detected globally, while transcripts for glc1.8/glc3.2 were detected primarily in haemocytes and the fat body (Fig. 1c).

Transcripts were detected in multiple tissues for a majority of the 21 predicted single-copy genes, but very weak or no amplicons were detected for orph-E3, orph-M1, orph-M2, orph-M3 and orph-N1 (Fig. 1d). While orph-M5 was detected in all host tissues, most of the other single copy genes expressed in \( P. \) includens appeared to be preferentially expressed in a subset of tissues. For example, orph-G1 and orph-G2 were most prominently detected in fat body and salivary glands, while orph-I2 and orph-L1 were most...
**Fig. 1.** RT-PCR analysis of *ptp* (a), *ank* (b), *glc1.8* and *egf1.0* (c) and single-copy (*orph*) gene (d) expression in selected tissues from *P. includens* larvae at 24 h post-infection. Host tissues examined for each gene were haemocytes, fat body, gut, nervous system and salivary gland. Amplification of the *P. includens* 18S rRNA from each tissue is shown in (c), which served as a loading control.
strongly detected in haemocytes (Fig. 1d). Other single-copy genes, however, like *orph-B1* and *orph-E1* were detected at similar levels in haemocytes, the fat body and gut with little or no expression apparent in nervous tissue or salivary glands (Fig. 1d).

**Spatial patterns of gene expression in hosts remain constant but transcript abundance tends to decline with time**

We followed our RT-PCR studies with a relative quantitative RT-PCR (rqRT-PCR) analysis of a subset of MdBV genes (*ptp-H2, ptp-J1, ank-H4, ank-N5, glc1.8/3.2, egf1.0/1.5 and *orph-B1*) in host haemocytes, fat body and gut over the 6 day period naturally required for parasitism of *P. includens* by *M. demolitor*. The genes selected for this analysis included particular members of each gene family plus one single-copy gene. These genes also exhibited a range of expression patterns in our RT-PCR analysis, with some appearing to be preferentially expressed in a particular tissue (*ptp-H2* and *ptp-J1*), and others being detected in multiple tissues (*ank-H4, ank-N5, glc1.8/3.2, egf1.0/1.5* and *orph-B1*). Overall, our results corroborated the spatial patterns of expression detected for these genes by RT-PCR at 24 h post-infection (Fig. 2). They also indicated that relative transcript abundance for most genes declined between 24 and 120 h (Fig. 2). For example, our RT-PCR data suggested that *ptp-H2* was preferentially expressed in host haemocytes at 24 h post-parasitism (Fig. 1). Our rqRT-PCR data confirmed that transcript abundance for *ptp-H2* at 24 h post-infection was highest in haemocytes and essentially non-detectable in fat body and gut, while also showing that transcript abundance was lower thereafter in haemocytes and remained undetectable in the other tissues. Reciprocally, our RT-PCR data indicated that *ank-H4* was expressed in all host tissues at 24 h (Fig. 1); our rqRT-PCR data supported this finding while also indicating that transcript abundance was greater at 24 h in the fat body than in haemocytes or the gut (Fig. 2). Thereafter, however, the relative transcript abundance of *ank-H4* declined in each host tissue, as seen with *ptp-H2* (Fig. 2). Inspection of the data for *ank-H5, glc1.8/3.2* and *egf1.0/1.5* revealed similar trends of declining transcript abundance in host tissues with time after infection (Fig. 2). The exception to this trend was *orph-B1*, where transcript abundance was similar in host haemocytes and fat body between 24 and 120 h post-infection (Fig. 2).

**Some MdBV genes are expressed in M. demolitor**

As with *P. includens*, we first conducted RT-PCR assays to determine whether any genes in the encapsidated genome of MdBV were expressed in *M. demolitor*. By using total RNA from 2-day-old adult wasps as template, we weakly detected amplicons for only three genes in males (*ptp-H4, ptp-H5* and *ank-N5*), whereas in females we detected amplicons for five *ptp* family members (*ptp-H5, ptp-J1, ptp-J3, ptp-J4* and *ptp-N1*), seven *ank* family members (*ank-F5, *ank-G3, ank-G4, ank-H4, ank-I1, ank-N1* and *ank-N5*), *glc1.8/3.2* and eight single-copy genes (*orph-A1, orph-A2, orph-B1, orph-D2, orph-D3, orph-G1, orph-G2* and *orph-I2*) (Fig. 3). We considered the possibility that preferential expression of these genes in females could reflect activity associated with replication or that virions in the calyx could be a source of contaminating DNA template. However, results using template prepared from only the head and thorax of females appeared overall to be similar to the whole-body samples (Fig. 3). We also detected no amplicons in either adult males or females when template from whole-body samples was not reverse transcribed (data not shown).

Focusing on those genes in which transcripts were detected in adult females, we also examined expression in 24 h *M. demolitor* eggs, last instar larvae, male pupae and female pupae. Amplicons for *ank-H4* were detected in each of these life stages (Fig. 4a). In contrast, we weakly detected an amplicon for *ptp-J1* in the egg stage and amplicons for *orph-B1* and *orph-D3* in male and female pupae (Fig. 4a). Combined with our results from *P. includens*, these data identified *ank-H4* as the only encapsidated gene transcribed in most tissues of infected hosts and multiple life stages of *M. demolitor*. These results also indicated that *orph-B1* is expressed in selected host tissues (haemocytes and fat body) and a subset of wasp life stages (pupae and adults).

Differences in expression levels of our internal control (18S ribosomal gene) between *P. includens* and *M. demolitor* prevented comparison of relative transcript abundance between infected hosts and wasps. We therefore generated standard curves to quantify transcript copy number for *ank-H4* and *orph-B1* per 5 ng of total RNA from infected *P. includens* haemocytes and each life stage of *M. demolitor*. Our results indicated that copy number of *ank-H4* ranged from a low of 511±142 per 5 ng of RNA from eggs to a high of 1088±277.6 in adult female wasp abdomens (Fig. 4b). However, these values were also much lower than the 12307±2692 copies of *ank-H4* per 5 ng of RNA from *P. includens* haemocytes. Fewer than 50 copies per 5 ng of RNA of *orph-B1* were detected in *M. demolitor* eggs, larvae and pupae (Fig. 4b). In contrast, 2343±285 copies of *orph-B1* per 5 ng of RNA were detected in adult female abdomens, which was greater than the 1611±50 copies of *orph-B1* detected in host haemocytes (Fig. 4b).

**DISCUSSION**

Successful parasitism of hosts by *M. demolitor* fully depends on co-infection by MdBV (Strand & Noda, 1991; Strand et al., 1992; Pruijssers et al., 2009). Here, we show that most predicted ORFs in the encapsidated genome of MdBV are transcribed in infected *P. includens*, which also suggests they have functional roles in parasitism. Prior studies provide insights into the activity of some gene-family members. For example PTP-H2 is a functional tyrosine phosphatase that, together with Glc1.8, inhibits...
adhesion of haemocytes to *M. demolitor* and other foreign targets (Beck & Strand, 2003, 2005; Pruijssers & Strand, 2007; Suderman *et al.*, 2008; Eum *et al.*, 2010). Other studies indicate that Ank-H4 and Ank-N5 are functional IxBs, while Egf1.0 and Egf1.5 are secreted proteins that disable melanization of host haemolymph by inhibiting the activation of prophenoloxidase (Thoetiattikul *et al.*, 2005; Beck & Strand, 2007; Lu *et al.*, 2008, 2010). In contrast, the function of other members in these gene families and of the single-copy genes MdBV encodes are unknown.

**Fig. 2.** rqRT-PCR analysis of *ptp-H2, ptp-J1, ank-H4, ank-N5, glc1.8/3.2, egf1.0/1.5 and orph-B1* in *P. includens* haemocytes, fat body and gut at 0–1, 24, 72 and 120 h post-infection by MdBV. The haemocyte sample at 24 h post-infection was standardized to a value of 1. Transcript levels for the other tissue samples and time points were then expressed as an increase or decrease relative to the 24 h control. Each treatment and time point was measured three times by using independently collected samples. Error bars indicate SEM. Different letters above a given bar indicates that transcript abundance significantly differs.

**Fig. 3.** RT-PCR analysis of *ptp* (a), *ank* (b), *glc1.8* and *egf1.0* (c) and single copy (*orph*) gene (d) expression in 2-day-old adult male and female *M. demolitor*. Total RNA isolated from whole bodies of adult male wasps, whole bodies of adult female wasps or the head and thorax only of adult females was used as template for each MdBV gene examined. Amplification of the *M. demolitor* 18S rRNA from male and female wasps is shown in (c), which served as a loading control.
In Table 1, we summarize our overall results by indicating which MdBV genes are expressed in *P. includens* and which are expressed in one or more life stages of *M. demolitor*. These data collectively suggest the following trends. In hosts, most *ptp* family members appear to be expressed in more than one host tissue. However, some family members also appear to be preferentially expressed in particular tissues, which suggests they interact with organ- or cell-specific physiological processes. Thus for example, the specific expression of *ptp-H2* and preferential expression of *ptp-J1* in haemocytes is consistent with the suggestion that both family members are likely to interact with immunological or other functions regulated by haemocytes. Sequence analysis further suggests that only four other family members (*ptp-H3, ptp-H5, ptp-N1* and *ptp-N2*) besides *ptp-H2* encode functional tyrosine phosphatases, whereas other family members are pseudophosphatases whose functions could include functions as substrate traps or binding proteins. Thus, in the case of *ptp-H2* and *ptp-J1*, the former is a functional *ptp* whose activity is restricted to infected host haemocytes while the latter is a pseudophosphatase whose currently unknown function is also likely to involve interaction with processes that take place primarily in immune cells.

Most *ank* family members are also expressed in multiple host cells or organs but relatively few appear to be preferentially expressed in a particular tissue (*ank-F4 and ank-N1*). Instead, several (*ank-F5, ank-H4, ank-I1, ank-N4 and ank-N5) appear to be expressed at similar levels in two or more organs. Thus, it is possible that *ank* family members have evolved to interact with processes and/or pathways that operate in multiple tissues. This finding is fully consistent with experimental data for *ank-H4* and *ank-N5*, given that insect NF-κB signalling pathways operate in diverse tissues (Aggarwal & Silverman, 2008; Uvell & Engström, 2007). Global expression of *egf1.0/1.5* is also consistent with these genes encoding secreted proteins whose only known function is inhibition of haemolymph melanization (Lu et al., 2010). On the other hand, we were surprised to detect expression of *glc1.8* in tissues other than haemocytes, which suggests this gene may have functions in addition to disrupting adhesion of immune cells. The absence of expression for several predicted single-copy ORFs also suggests they are pseudogenes in the process of being lost from the genome.

The encapsidated genomes of BVs from wasps in the same or closely related genera share several single-copy genes and gene families, whereas isolates from more distantly related genera share few or no genes (Espagne et al., 2004; Provost et al., 2004; Webb et al., 2006; Falabella et al., 2007; Annaheim & Lanzrein, 2007; Weber et al., 2007; Desjardins et al., 2008; Choi et al., 2009). Thus, the only genes shared among BV isolates from wasps in the subfamilies Microgastrinae and Cardiochilinae are members of the *ptp* and *ank* families, while BVs from microgastrine and cardiochiline wasps appear to share no genes with BVs from chelonines.
Comparing our results to other microgastrines and cardiochilines, we note that 22 members of the *ptp* family from CcBV, nine members of the *ptp* family from GiBV and seven members of the *ptp* family from *Toxoneuron nigriceps* (TnBV) also exhibit variable patterns of expression, with a majority of family members being expressed in

**Table 1. MdBV transcriptome activity in *P. includens* and adult *M. demolitor***

<table>
<thead>
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<th>Gene</th>
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<td><em>glc-1.8/3.2</em></td>
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a subset of host tissues (Chen et al., 2003; Provost et al., 2004; Gundersen-Rindal & Pedroni, 2006). In contrast, Ibrahim et al. (2007) report that all 14 members of the ptp family from CpBV are expressed globally or near globally in the host Plutella xylostella. Variable patterns of expression are also reported for ank genes from TnBV and CpBV (Falabella et al., 2007; Bae & Kim, 2009). Similar to MdBV, data for selected genes from other microgastrines and cardiochilines also show a tendency for expression to begin within hours after host infection, followed by a rapid or progressive decline over subsequent days (summarized by Webb & Strand, 2005; Strand, 2010). In contrast, studies of the cheloneine wasp Chelonius inanitus indicate that some genes encoded by CiBV are expressed persistently while others are expressed at particular times post-infection at predominantly low levels (Johner & Lanzrein, 2002; Bonvin et al., 2005; Weber et al., 2007).

Recent studies have identified several wasp and proviral genes likely to have roles in replication of PDVs in calyx cells (Bézier et al., 2009; Volkoff et al., 2010; Wetterwald et al., 2010). In contrast, little is known about whether genes packaged into the encapsidated genomes of PDVs are also expressed in wasps. Our results indicate that most MdBV genes are not expressed in M. demolitor, although we do detect expression of selected ptp- and ank-family members, and orphan genes in adult females and/or other life stages (Table 1). Our results further suggest that expression of some of these genes is not strictly associated with activity in calyx cells because the same transcripts are detected in samples prepared from only the head and thorax of adult females. However, the expression patterns of other genes, like orph-B1, do suggest activity is preferentially associated with replication, because transcript abundance is much higher in the female abdomen than in thorax-plus-head samples. Studies with other BV-carrying wasps as well as our own observations with M. demolitor indicate that excision and packaging of viral DNAs into capsids begins during the late pupal stage (day 3 in M. demolitor) and continues during the adult stage (Bézier et al., 2009; Wetterwald et al., 2010; M. R. Strand, unpublished observations). It is also known that BV DNAs packaged into virions are excised and/or amplified in other wasp tissues during the late pupal and adult stages of females or both sexes, although no virions are produced (Gruber et al., 1996; Savary et al., 1999). Whether the transcripts we detect in adult female M. demolitor are linked to excision and/or amplification is unknown. It is also unclear whether any of these transcripts are translated. However, given the evidence for the 1aB activity of ank-H4, it will be very interesting to determine whether this gene has the same function in M. demolitor. A second ank family member of interest is ank-G3, which was the only MdBV gene for which we detected expression in female wasps but did not detect any expression in P. includens (Table 1).

Outside of our current results, the only other data documenting expression of encapsidated PDV genes in wasps derive from IVs. One structural gene, designated p12, from CsIV resides on a genomic segment packaged into virions, which is also expressed in females during replication (Deng & Webb, 1999). Expression of selected members of the CsIV rep and inex gene families are also detected in female pupae and adults of C. sonorensis and Transarea rostrale (Theilmann & Summers, 1986; Hilgarth & Webb, 2002; Turnbull & Webb, 2002; Rasoolizadeh et al., 2009). In the case of BVs, preliminary observations reported by Provost et al. (2004) indicate that selected ptp family members from CcBV are expressed in adult C. congestata, but whether expression is restricted to adult females, as observed for MdBV, remains unclear.

METHODS

Insects. M. demolitor and P. includens were reared at 27 °C with a 16 h light/8 h dark photoperiod as previously described (Strand, 1990; Strand & Noda, 1991). Wasps preferentially parasitize third instar P. includens with a single offspring per host, developing from an egg to last (fourth) instar larva in 6 days (120 h). The wasp larva then chews its way out of the host’s body on day 7 to pupate within a silken cocoon followed by emergence of the adult 4 days later. The host, in contrast, dies 1–2 days after the wasp larva emerges.

Virus collection and injection into P. includens larvae. MdBV was collected from adult female wasps as previously described (Beck & Strand, 2003; Strand et al., 1992). As is conventional in the PDV literature, the amount of MdBV collected from the reproductive tract of a single adult female is defined as one wasp equivalent. MDBV packages only one genomic segment per virion and the 15 segments comprising the genome are non-equimolar in abundance (Beck et al., 2007). Prior studies determined that one wasp equivalent on average contains 1 × 1010 virions and that M. demolitor injects 0.01–0.1 wasp equivalents into third-fourth instar hosts (Beck et al., 2007). For this study, we injected 0.05 wasp equivalents of MdBV into day 1 third instar P. includens using a glass needle and micromanipulator as previously described (Beck et al., 2007).

MdBV genomic segment and predicted-gene nomenclature. The encapsidated genome of MdBV was previously deposited in GenBank under accession numbers AY887894, AY875680–AY875690, AY848690, AY842013 and DQ002040 (Webb et al., 2006). The 15 genomic segments are named by upper-case letters from the smallest (genomic segment A 3433 bp) to largest (genomic segment O 34 355 bp), while most predicted genes are named by their location on a given genomic segment. Thus, members of the ptp gene family consist of one predicted gene located on genomic segment D (ptp-D1), five on segment H (ptp-H1, 2, 3, 4 and 5), four on segment J (ptp-J1, 2, 3 and 4), and three on segment N (ptp-N1, 2 and 3) (Webb et al., 2006; Prijiers & Strand, 2007). Members of the ank gene family are named similarly (ank-C1, C2, F4, F5, G3, G4, H4, I1, J4, N1, N4 and N5), whereas all egf (egf0.4, egf1.0 and egf1.5) and gcl genes (gcl1.8 and gcl3.2) reside on genomic segment O and are named by the size of their corresponding cDNAs (Strand et al., 1997; Trudeau et al., 2000; Webb et al., 2006). As previously noted, most predicted single-copy genes are orphans that are named by their location on a given genomic segment (orph-A1, A2, B1, C3, D2, D3, D4, E1, E2, E3, F1, G1, G2, I2, K1, L1, M1, M2, M3, M4, M5 and N1) (Webb et al., 2006).
and salivary glands pooled from four larvae. Haemocytes were collected by bleeding four larvae from a cut proleg into PBS. RNA pools from wasps consisted of: (i) 200 M. demolitor eggs (6 h old), (ii) three third-instar larvae collected from parasitized P. includens, (iii) three male and female M. demolitor pupae (2 days old) collected from dissected cocoons, or (iv) three male and female adult wasps (2 days old) collected from the general laboratory culture. For some assays, adult female wasps were also divided into head-plus-thorax and abdomen-only samples. Total RNA was then isolated from each of these samples using a High Pure RNA Isolation kit (Roche) according to the manufacturer’s instructions. Quantification of RNA was done using a Nanodrop spectrometer (Thermo Scientific).

For first-strand cDNA synthesis, 100 ng of total RNA was reverse transcribed in 20 μl reactions by using random hexamers and Superscript III (Invitrogen). RT-PCRs were run using a Bio-Rad thermocycler and 25 μl reaction volumes containing 1 μl of cDNA and 2.5 μM of appropriate gene-specific primers. Primers used to amplify each ptp family member were described by Pruissers & Strand (2007), while primers used to amplify gcl1.8 and egf1.0 were described by Beck et al. (2007). Primer pairs used to amplify ank family members and predicted single copy (orph) genes were designed from the sequence of the corresponding ORF in the encapsidated genome (Supplementary Table S1, available in JGV Online). Reaction cycling conditions were as follows: initial denaturation at 94 °C for 2 min followed by 35 cycles at 94 °C for 20 s, annealing at 50 °C (ptp, gcl, egf and single-copy genes) or 55 °C (all ank family members) for 10 s, extension at 65 °C for 30 s and a final extension at 72 °C for 7 min. 18S rRNA from P. includens (GenBank accession no. AY298945) was used as an endogenous control (Pruissers & Strand, 2007).

**rqRT-PCR and quantitative-PCR (qPCR) analyses.** rqRT-PCR reactions were run using cDNA templates prepared from haemocytes, fat body and the gut of infected larvae as described above. A total of three infected larvae were dissected for each tissue and time point (1, 24, 72 and 120 h). The reactions for all samples were run using a Rotor-Gene 3000 Cycler (Corbett) and 10 μl reaction volumes containing 1 μl of cDNA, 5 μl of iQ SYBR Green Supermix (Bio-Rad) and appropriate gene-specific primers. Primers used in real-time assays to amplify ptp-H2, ptp-J1, gcl1.8 and egf1.0 were identical to the primers used in the RT-PCR assays (see above). Primers used to amplify ank-H4, ank-N5 and orph-B1 in real-time assays are presented at the bottom of Table 1. Amplification of P. includens 185 rRNA served as an internal control. Cycling conditions were: initial denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 50 °C for 15 s and extension at 72 °C for 20 s. Data were acquired during the extension step and analysed with the Rotor-Gene application software. For every primer set, a standard curve was constructed using gene-specific primers and known amounts of plasmids containing cloned ank-H4 and orph-B1 cDNAs as template. The copy number of each transcript in 5 ng of total RNA from three independent wasp and host samples was then determined as previously described (Beck et al., 2007) followed by ANOVA and Tukey–Kramer HSD tests. All statistical analyses were performed using the JMP 7.0 platform (SAS).

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**REFERENCES**


