Polydnaviruses: Evolution and Applications

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Abstract Polydnaviruses ("poly" referring to the poly-dispersed DNA segments) (PDVs) are the unique viruses, which are obligatory symbionts with parasitoid wasps (the primary host). Polydnaviridae was formally recognized as a family of viruses in 1991. Corresponding to the PDV-carrying wasp families, Braconidae and Ichneumonidae, PDVs are subdivided into two genera, Bracovirus (BV) and Ichnovirus (IV). The PDV genome is integrated into the wasp's chromosome as a provirus and vertically transmitted through wasp germ lines. PDV virions only replicate in the calyx cells of female wasps, which are injected into caterpillar hosts (the secondary host). PDV genes are expressed in the secondary host, which suppress the host's immune system, prevent encapsulation, and regulate the host's physiology to facilitate parasitism; this results in the death of the secondary host. A breakthrough study on the wasp transcriptome showed that BVs evolved from a nudivirus, while IVs originate from a different virus ancestor that belongs to a new virus family. Due to PDV gene function, PDV-associated gene products are also used for pest control in crops. In this chapter, the evolution, life cycle, functional genes and applications of PDVs will be reviewed.

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1   Introduction

Polydnaviruses (PDVs), i.e. double-stranded DNA viruses with segmented genomes, are unique insect viruses that exist in obligate association with certain parasitic wasps in mutualistic symbioses. They have a unique life cycle between their primary host (parasitoid wasps) and secondary host (lepidopteran insects). PDVs occur as proviruses integrated in the wasp genome. During oviposition, the female wasp produces virion particles and injects them into the wasp's lepidopteran host, with wasp eggs. The PDV genes expressed in the secondary host to lead to parasitoid survival. PDVs replicate exclusively in parasitoid wasp ovaries, and the gene products in the secondary host can regulate host physiology and suppress the host's immune system to facilitate parasitism. This also ensures the vertical transmission of the PDV genome to the next wasp generation (Béliveau et al. 2015).

According to the parasitoid wasp species families Ichneumonidae and Braconidae, PDVs are divided into two genera, bracoviruses (BVs) and ichnoviruses (IVs), respectively. BVs are associated with wasp species comprised of six subfamilies, and are estimated to contain >50,000 species (Strand and Burke 2019). IVs have so far been observed associated with only two subfamilies, Campopleginae and Banchinae, which contain >13,000 species (Gundersen-Rindal et al. 2013). Also, a virus group associated with ichneumonid wasps of banchinae subfamily has been proposed as a third taxonomic genus of Polydnaviridae, based on the study of PDVs from the wasp *Glypta fumiferanae* (Lapointe et al. 2007). This PDV is different from Campoplegine IVs with different virion morphology, genes coding for protein tyrosine phosphatases shared with BVs, and different wasp lineages (Lapointe et al. 2007).

The main function of PDV genes is to prevent wasp egg encapsulation by the host's immune system. PDV-mediated suppression of the host's immune system was first reported by Edson, who showed that *Campoletis sonorensis* ichnovirus (CsIV) gene expression is essential for preventing *C. sonorensis* egg encapsulation (Edson et al. 1981). Subsequently, a number of PDVs genes that interact with the parasitoid's immune system have been studied. Some of them are formed into families such as the Glc family (Johnson et al. 2010), protein tyrosine phosphatase (PTP) family (Pruijssers and Strand 2007), Anks family (Beck and Strand 2007) and Egf family (Lu et al. 2008). Other genes exist as a single copy, such as CrV1 (Asgari et al. 1997), CrV2 (Cooper et al. 2011) and CCV1 (Labropoulou et al. 2008). Parasitoids are able to disrupt host insect development and result in the mortality of the host. Functional genes of PDVs open new ways to pest biological control in agriculture.

2 Where Did the PDVs Come from?

The study of PDVs started in the late 1960s (Salt 1965). In the early 1970s, these particles were identified in the ovaries of certain wasps through electron microscopy (Volkoff et al. 2010). Polydnaviridae was formally recognized as a family of viruses in 1991 (Francki et al. 2012). Early morphological research showed that BV and IV virions are similar to viruses from the family Baculoviridae and Ascoviridae, respectively (Bigot et al. 2008; Federici and Bigot 2003; Stoltz et al. 1976). However, further research noted that PDVs neither share any significant homologous genes with baculovirus or ascovirus nor share homology with other viral genes that have predicted function in DNA replication, transcription or virion formation (Strand and Burke 2012). Therefore, two models have been suggested to answer questions related to PDV evolution. One is that PDVs evolved from a wasp ancestor, and during prolonged evolution obtained the ability to produce and package circular DNA that contains wasp genes encoding proteins that withstand the immune responses of the insect host (Espagne et al. 2004; Schmidt et al. 2005; Whitfield and Asgari 2003). Another model proposes that PDVs evolved from insect DNA viruses associated with the ancestor of campopleginae and microgastroid wasps and developed a beneficial association. The PDVs integrated into the wasp genome and lost viral replication and structural genes and acquired virulence genes from the wasp. PDVs are not viruses, but rather wasp organelles that evolved from a virus (Federici and Bigot 2003). PDVs have virus-like features, such as viral particle assembly and infectibility in wasp hosts. However, PDVs are also not like other known viruses due to their unique attributes, such their large segmented circular dsDNA genomes, their ability to act as beneficial symbionts in one host (wasps) and pathogens in another (the hosts of wasps), and genome replication and gene expression in different hosts (Strand and Burke 2019).

The advanced demonstration of the origin of these particles was confirmed with developments in DNA sequencing technologies and mass spectrometry, which have allowed the analysis of PDV genomes and protein components (Béliveau et al. 2015; Volkoff et al. 2010). Currently, nine PDV genomes have been sequenced: *Cotesia congregata* BV (CcBV), *Microplitis demolitor* BV (MdBV), *Cotesia plutellae* BV (CpBV), *Glyptapanteles flavicoxis* BV (GfBV), *Glyptaanteles indiensis* BV (GiBV), CsIV, *Tranosema rostrale* IV (TrIV), *Hyposoter fugitivus* IV (HfIV) and *Glypta fumiferanae* IV (GfIV) (Lapointe et al. 2007; Espagne et al. 2004; Webb et al. 2006; Desjardins et al. 2008; Tanaka et al. 2007; Chen et al. 2011). All genome data of PDV viruses share common features, such as large genomes (from 189 to 606 kb), low coding densities (17–33%), many genes organized into gene families, and numerous genes that share homology with the genes of wasps or other eukaryotes. BVs and IVs rarely share genes with each other, which indicates that their evolution was independent.

2.1 BVs Evolved from a Betanudivirus

Within Braconidae, six subfamilies form the microgastroid complex, which diverged approximately 100 million years ago (Mya) from other braconid subfamilies without BVs. Based on this phylogeny, it suggested that all BV-carrying species originate from a common ancestor (Strand and Burke 2019). The first breakthrough came from a study on the transcriptome in ovary DNA libraries of two braconid wasps (*C. congregate* and *Chelonus inanitus*) (Bézier et al. 2009). Complementary genomic and proteomic analysis showed that several genes typical of nudiviruses were expressed in ovaries in bracovirus-associated species during replication (Wang and Jehle 2009).

Nudiviruses form a sister taxon to the Baculoviridae, which is an extensively studied insect family used for biological control of lepidopteran pests, such as *Autographa californica multinucleopolyhedrosis* virus (AcMNPV) (Rohrmann 2014). The divergence of nudiviruses and baculoviruses dates back to 300 Mya, while nudiviruses and bracoviruses diverged approximately 100 Mya (Strand and Burke 2012; Thézé et al. 2011). Mass spectrometry analysis (LC MS-MS) of purified particles showed that the nudivirus-like genes that are expressed in BV-carrying wasps encode viral particle components (Bézier et al. 2009). The involvement of nudivirus genes in virus particle production was also confirmed by functional analyses with RNAi knockdown methods (Burke et al. 2013). Moreover, homologs of nudiviral genes were isolated successfully by PCR with primers designed for two nudiviral genes (HzNVorf9-like1 andHzNVorf128-like), well-conserved in *C. congregate* and *C. inanitus*, in species from a series of BV-associated wasps. These results strongly suggest that they were inherited from a common ancestor (Bézier et al. 2009). This ancestral wasp lived approximately 100 million years ago based on phylogenetic analysis (Murphy et al. 2008). The ancestor virus could belong to the genus Betanudivirus according to the phylogenetic analysis (Thézé et al. 2011).

2.2 IVs Evolved from Viruses that Have Not Yet Been Described

The transcripts of the ichneumonid wasp *Hyposoter didymator*, gene organization within the wasp genome, and proteins associated with IV virus particles were analyzed. Unlike braconids, no nudivirus or baculovirus-like genes have been identified (Volkoff et al. 2010). Some domains of the genomes which contain the genes coding for structural proteins in IV virions were found. These genes have virus-like features and form clusters in the genome of the wasp, which indicate that IVs originated from a virus, whereas these regions share no homologous genes with other virus families. The genomic regions encoding genes involved in IV virus particle production, which exist in the gene-rich regions of wasp chromosomes,

were named ichnovirus structural protein encoding regions (IVSPER). Until now, three IVSPERs have been identified for IVs, and two are located near a sequence segment corresponding to virus assembly. The gene content of the three IVSPERs belongs to seven gene families. Homologs of IVSPER genes have been found in *T. rostrale* by blast searches of an expressed sequence tag (EST) database (Volkoff et al. 2010) and were identified in an ovarian cDNA library derived from *T. rostrale*. IVSPERs have a high density of genes lacking introns, suggesting an endogenized virus origin. Most IVSPER genes lack similarities with any other currently known viral genes and thus cannot be used for identification of the IV ancestor, which suggests that IVs evolved from a virus ancestor that has not yet been characterized (Volkoff et al. 2010).

IVs from subfamily Banchinae are different from those in Campopleginae wasps in their particle morphology and gene content (Lapointe et al. 2007), which raises the question as to whether these two IV groups had a common virus ancestor or not. Several analyses have shown that IV particles from both subfamilies are produced by IVSPER genes that are conserved between Banchinae and Campoplegine wasps (Béliveau et al. 2015; Volkoff et al. 2010). Two hypotheses have been proposed to explain this. First, IV may have originated from a single viral ancestor followed by virus gene loss; second, IVs may have originated from two independent viruses of the same family that occurred in both wasp subfamilies (Béliveau et al. 2015).

3   The Life Cycle of PDVs

PDVs have an obligate association with thousands of species of parasitic wasps in the families Braconidae and Ichnomonidae as they persist as an integrated provirus in the germline and somatic cells of associated parasitoid Hymenopteran wasps. The PDV life cycle occurs in two hosts, in which PDV replicates and transmits in the wasp host and replication specifically occurs in the calyx cells of pupal and adult stage female wasp ovaries (Gundersen-Rindal et al. 2013) (see Fig. 1). Following excision of the proviral DNA from wasp chromosomes, encapsidated virions accumulate at high concentrations in the lumen of late oviducts (Belle et al. 2002; Fleming and Summers 1991; Gruber et al. 1996; Xu and Stoltz 1991). The assembled virions contain multiple circular dsDNAs with large aggregate sizes, from 190 to 730 kb. The PDV pathogenic step occurs in the second host. At oviposition, the wasp injects one or more eggs into the lepidopteran host with a number of virions, ovarian proteins and venom secretions from the venom gland (Schmidt et al. 2001). PDV virions rapidly infect the host's hemocytes, fat body and other tissues, express virulence genes, and integrate their DNA segments into the genome of infected host cells (Strand and Burke 2014). Viral gene expression can be detected as early as 1 h after parasitism and continues throughout wasp larval development. These gene products have two main functions. First, they suppress the host's humoral and cellular immunity, which prevents egg encapsulation and mobilizes host protein stores to support parasitoid growth. Second, they regulate the host
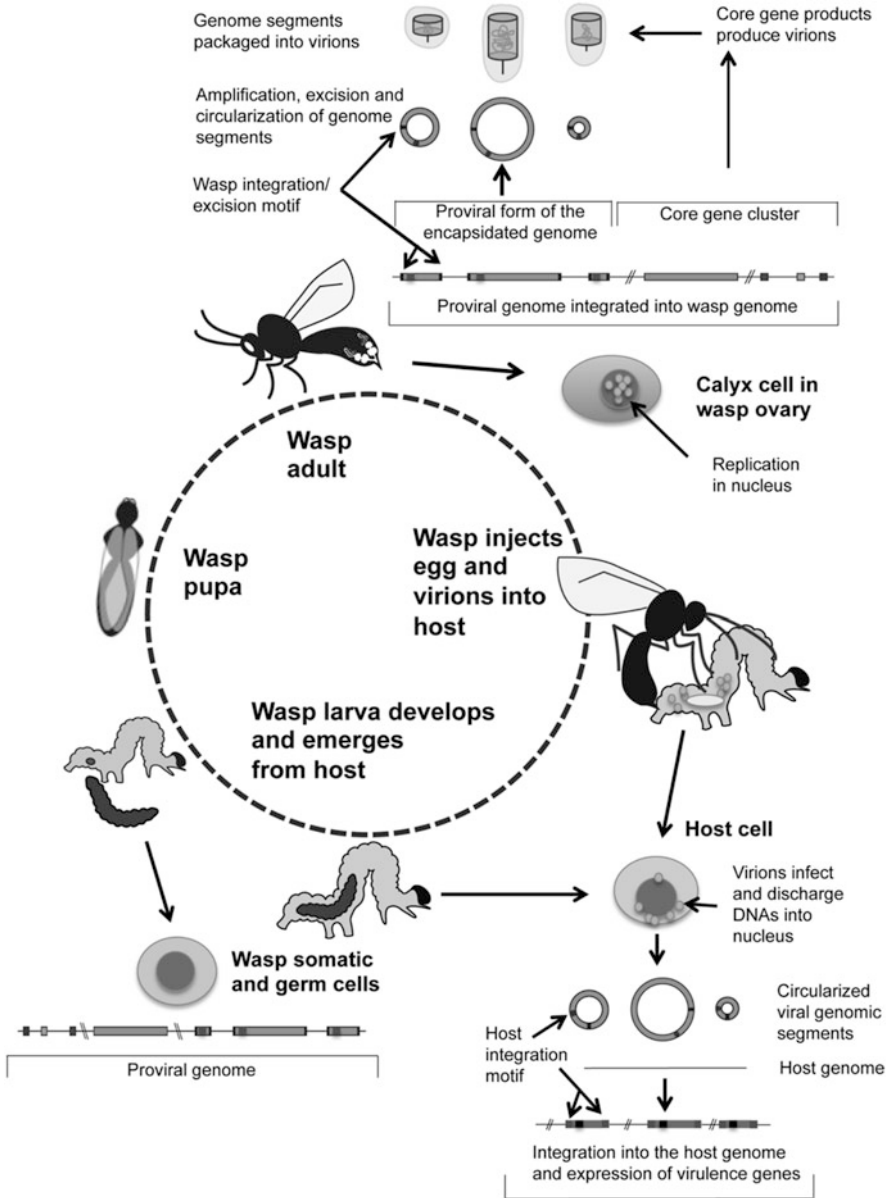
Fig. 1 Life cycle of PDV. (From Strand MR, Burke GR., PLoS Pathog 8(7): e1002757, 2012. With permission.)

physiology, which facilitates wasp offspring development, leads to parasitism success and results in the death of the host (Chevignon et al. 2014, 2015). The parasitoid wasp completes its life cycle inside the lepidopteran larva and emerges as an adult wasp while the host larva dies.

PDVs exist in two different forms, one of which is a provirus integrated into the parasitoid wasp's chromosome and the other exists as double-stranded circular DNA segments which are assembled into virions. Unlike viruses in any other family, PDVs cannot replicate in the wasp host because the genes for viral replication machinery and structural proteins that are required to produce virus particles are not assembled into virions, but are rather integrated into the chromosome of the parasitoid wasp. PDV are only vertically transmitted as integrated proviruses through the germline of wasps (Burke et al. 2013). During evolution, PDV proviral genomes have divided into two functional units. One includes genes (such as structural genes) with replication function, which are expressed but are not encapsidated into virions; the other includes multiple DNA domains including virulence genes, which are amplified and assembled into virions (Strand and Burke 2014).

The main function of PDV genes is to prevent wasp egg encapsulation by the host's immune system. The host insect's immune system includes cellular and humoral components. The cellular immune system refers to a response mediated by hemocytes, such as nodule formation, encapsulation and phagocytosis (Strand 2008). Hemocytes are composed of plasmatocytes, granulocytes, spherule cells and oenocytoids, which are classified by morphological, functional and molecular markers (Strand 2008; Nakahara et al. 2009). Granulocytes are responsible for non-self recognition and phagocytosis, while plasmatocytes form a capsule around foreign objects to induce encapsulation. Oenocytoids can express phenoloxidase and other components of the PO cascade to start melanization, while the function of spherule cells is not yet clear (Beck et al. 2011). Humoral defenses involve molecules produced by hemocytes or tissues with the ability to kill foreign objects (Kanost and Gorman 2008). Three classes of innate humoral responses have been defined, including defensive melanization, sentinel molecule binding and the induction of antimicrobial peptides (Gillespie et al. 1997).

The most important immune response of lepidopteran insects toward the eggs of parasitoids is encapsulation (Strand 2008). This response, involving the action of hemocytes, is mediated by two kinds of recognition receptors. The first kind is cell surface receptors, which are expressed on the surface of hemocytes, such as scavenger receptors, integrins and the nimrod superfamily (Kocks et al. 2005; Moita et al. 2005; Wertheim et al. 2005; Somogyi et al. 2008). The second kind is humoral pattern recognition receptors, such as lipopolysaccharide-binding protein, hemolin, soluble peptidoglycan recognition proteins, immunolectins, complement-like thioester-containing proteins and Gram-negative bacteria recognition proteins (Moita et al. 2005; Levashina et al. 2001; Irving et al. 2005; Dong et al. 2006; Ling and Yu 2006; Terenius et al. 2007). Encapsulation usually starts 2–6 h after parasitism and is accomplished by 48 h (Strand 2008).

4 PDV Genes that Interact with the Host Immune System

The key function of most PDV gene products is to disrupt host cellular and humoral immunity, including cytoskeleton degradation, adhesion disruption and hemocyte inactivation (Asgari and Schmidt 2004a). PDVs encode many genes, some of which form multimember families and while others exist as a single copy. Here, we have reviewed some important gene families and single genes in PDVs.

4.1 Cysteine (Cys) Motif Family

Cys-motif coding genes are detected in BVs and IVs. Each gene of this family has a variable cys-motif but shares a very conserved exon-intron structure, hydrophobic signal peptide and N-terminal glycosylation site (Dupas et al. 2003). The gene Mbcrp1 from *Microplitis bicoloratus* bracovirus (MbBV) contains a cysteine-rich trypsin inhibitor-like (TIL) domain coding sequence and the expression of recombinant MbCRP1 inhibits the expression actin in Hi5 cells, suggesting that expression of MbCRP is related to the disruption of the actin cytoskeleton (Luo and Pang 2006). Cys-motif proteins VHv1.1 and Vhv1.4 in CsIV are expressed in the fat boy and secreted into the hemolymph where they bind to the surface of hemocytes. VHv1.4 protein has been detected by immunoblot in the lepidopteran *Heliothis virescens* starting at 6 h post parasitization, and throughout the entire course of parasitism (Cui et al. 1997). In *H. virescens* larvae, VHv1.4 protein expressed from a recombinant baculovirus bound to granulocytes, which suggests that it has an important function in the suppression of the host encapsulation response (Cui et al. 1997). Another analysis of Cys-motif gene family gene transcripts from CsIV showed that WHv1.6 interacts with the cell membrane along with other organelles and prevents immunocytes from spreading or adhering to a foreign surface, which plays a major role in inhibiting the cellular encapsulation response by *H. virescens* (Gill and Webb 2013).

4.2 Glycosylated Central (Glc) Gene Family

The Glc gene family contains two identical genes (glc1.8) encoding for a cell surface mucin-like glycoprotein in MdBV. In parasitized hosts, the Glc1.8 protein localizes to the cell surface of MdBV-infected hemocytes and blocks both encapsulation and phagocytosis (Beck and Strand 2005). The expression of recombinant Glc1.8 in High Five cells as well as of S2 cells from *Drosophila melanogaster* greatly reduced phagocytosis ability, suggesting that this protein is involved in the suppression of the insect cellular immune response (Beck and Strand 2005). Similarly, knockdown of Glc1.8 by RNA silencing in MdBV infected cells reinstated normal adhesion and

phagocytosis ability (Beck and Strand 2003). Although Glc1.8 severely impaired adhesion and phagocytosis, it did not cause cell death (Beck and Strand 2003).

4.3 Epidermal Growth Factor (Egf) Family

The Egf family, which includes three members (egf1.0, egf1.5 and egf0.4), is encoded by MdBV. To date, only two PDV genes (ef1.0 and egf1.5) have been confirmed to inhibit the melanization of plasma from two permissive lepidopteran hosts (*Pseudoplusia includens* and *Helicoverpa zea*) and two non-permissive lepidopteran hosts (*Manduca sexta* and *Bombyx mori*) (Lu et al. 2010). Egf0.4 does not have anti-melanization activity (Beck and Strand 2007). The expression of egf1.0 and egf1.5 begins at about 2 h post parasitization and continues for over 7 days during the development of the parasitoid offspring. The MdBV Egf family encodes for small serine proteinase inhibitor (smapins) homologs that include a cysteine-rich trypsin inhibitor-like domain and function as competitive inhibitors of specific target enzymes (Zang and Maizels 2001). Further studies showed that egf1.0 inhibits the melanization of hemolymph in *M. sexta* by disabling the processing and catalytic activity of phenoloxidase activating proteinases1 (PAP1); the processing of pro-PAP1 and pro-PAP3 was inhibited (Lu et al. 2008). Efg1.5 shares an identical cys motif with efg1.0 but with an extended C-terminal repeat domain. Egf1.5 inhibited the processing and amidolytic activity of PAP1 and PAP3 in *M. sexta*, and moreover bound to PAP1, PAP3 and serine proteinase homolog 2 (SPH2) (Lu et al. 2010).

4.4 Ankyrin (Ank) Family

Ankyrin genes are present both in BVs and IVs, and have been named "IκB-like", "vankyrins", "cactus-like", "viral ankyrin" or "ank" genes (Huguet et al. 2012). A hypothesis has been proposed that PDV expression may disrupt the activities mediated by nuclear factor kappa-B (NF-κB) due to the presence of ankyrin genes in PDVs. NF-κB is a eukaryotic transcription factor that exists in the cytoplasm of cells in a dimeric inactive form bound to the inhibitor IκB. NF-κB transcription factors are key regulators of the immune responses of both insects and mammals. Further study showed that ankyrin proteins have the potential ability to disrupt NF-κB signaling in lepidopteran hosts, interfering with the regulation of apoptosis, elicitation of antiviral responses and antimicrobial peptide production (Fath-Goodin et al. 2009; Bae and Kim 2009; Shrestha et al. 2009; Falabella et al. 2007). Ank-H4 and Ank-N4 have been reported to disrupt Toll and imd signaling and antimicrobial peptide expression by binding to NF-κB (Thoetkiattikul et al. 2005). One member of the ank gene family encoded by TnBV has also been shown to interact with insect NF-κB and cause its retention in the cytoplasm, which may function to suppress the

immune response in parasitoid hosts (Falabella et al. 2007). Another ank gene interferes with cytoskeleton organization in *Drosophila* germline cells (Duchi et al. 2010). In Spodopteran hosts, an ank gene (Hd27-vank1) of *Hyposoter didymator* Ichnovirus (HdIV) with high expression suggests that Hd27-vank1 might have pleiotropic functions during the parasitism of these insect species (Clavijo et al. 2011). In *H. virescens*, ank family genes are expressed within 2–4 h post parasitization and reach peak levels by 3 days post parasitization (Kroemer and Webb 2005).

4.5   Cotesia rubecula *Polydnavirus Gene 1 (CrV1)*

CrV1, a gene product of *Cotesia rubecula* Bracovirus (CrBV), is the best characterized gene so far among the four gene products detected in *Pieris rapae* tissues. This gene was cloned and sequenced by Dr. Asgari (Asgari et al. 1996). The CrV1 gene product is a secreted glycoprotein expressed in the hemocytes and fat body cells of the secondary host *Pieris rapae*. This gene product has been implicated in the depolymerization of the actin cytoskeleton of host hemocytes and disruption of the capacity of hemocytes to spread onto foreign surfaces (Asgari et al. 1997). In *C. rubecula*, CrV1 is the only gene expressed in the host hemocyte. The expression of this gene lasts no more than 4–8 h after parasitization (Asgari and Schmidt 2002). Its function is transient and, after the appearance of the wasp, the cells return to normal function (Le et al. 2003). It has been shown that CrV1 is endocytosed or phagocytized by host hemocytes. Asgari and Schmidt found that a coiled-coil domain containing a putative zipper is required for CrV1 function and this domain affects the binding and uptake of the CrV1 protein by hemocytes (Asgari and Schmidt 2002). It is known that CrV1 binds to lipophorin, forming a complex with the lipid carrier (Asgari and Schmidt 2002; Glatz et al. 2004b). The CrV1 complex with lipophorin interacts with hemocytes, and is then taken up by lipophorin or scavenger receptors as part of a clearance reaction (Schmidt et al. 2005). Within the hemocyte, the complex interacts with membrane-anchored hemolin to inhibit its functions, including actin depolymerization and lipopolysaccharide binding and agglutination (Ye et al. 2018).

4.6   *Protein Tyrosine Phosphatase (PTP)*

PTP is a BV gene family that interrupts kinase/phosphatase cycles via the dephosphorylation of target proteins, thereby inactivating the signaling pathway (Eum et al. 2010; Serbielle et al. 2012). PTP transcripts were detected starting at 2 h post parasitization by quantitative polymerase chain reaction analysis (Q-PCR), for approximately 8 days, and the maximum expression occurred between 24 and 48 h post parasitization (Chen et al. 2003). PTP transcripts were detected in the

hemocytes, fat body and brain of parasitoid hosts (Chen et al. 2003). Some PTP genes encoded by MdBV were found to be expressed in prothoracic glands and the nervous system, which indicates that PTPs may be involved in disrupting the prothoracicotropic hormone signaling pathway (Pruijssers and Strand 2007). An alteration in the wasp host by PTP cannot be ruled out. Some PTP genes inactivate host hemocytes to suppress the immune system by regulating the actin cytoskeleton (Chen et al. 2003). Additionally, PTP-H2 of MdBV expressed in Sf21 cells causes apoptosis (Suderman et al. 2008), and further enzymatic analysis showed that PTP-H2 is a tyrosine phosphatase (Eum et al. 2010). Similarly, transient expression of CpBV-PTPs in *Spodoptera exigua* hemocytes caused reductions in cell spreading and encapsulation activities (Ibrahim and Kim 2008). Therefore, PTPs are involved in the suppression of multiple processes including development and immunity.

4.7 **Cotesia congregate** *Polydnavirus Gene 1 (CcV1)*

CcV1 gene is an orthologous gene of CrV1 and is expressed in the lepidopteran *M. sexta* parasitized by *C. congregata*. CcV1 transcripts are detected in the fat body and hemocytes within 2 and 4 h post parasitization, respectively, and the expression lasts for at least 48 h (Le et al. 2003). The expression of CcV1 has been observed from 24 h post parasitization in the hemocyte cytoplasm until the emergence of the wasp pupa (Amaya et al. 2005). Functional experiments showed that CcV1 interacts directly with hemolin and inhibits hemocyte function in the normal immune response (Labropoulou et al. 2008).

4.8 **Cotesia rubecula** *Polydnavirus Gene 2 (CrV2)*

The protein CrV2 is encoded by CrBV, which is expressed in the lepidopteran host *P. rapae* within 4–12 h post-parasitization. CrV2 with an ORF of 963 bp produces a glycoprotein of approximately 40 kDa and transcripts in hemocytes and fat body cells. CrV2 has a coiled-coil region, which may be involved in the formation of putative CrV2 trimers that are detected in the hemolymph of parasitized host *P. rapae* larvae (Glatz et al. 2004b). Further study showed that CrV2 protein specifically interacts with mammalian Gα subunits of heterotrimeric G-proteins using a time-resolved Förster resonance energy transfer (TR-FRET) assay. This result suggests that CrV1 may target Gα subunits to alter immune signaling pathways to regulate the insect hemocyte immune system, which may be developed for novel insect control strategies (Glatz et al. 2004b).

5 Polydnavirus Applications in Biological Control

As described above, PDV genes are used by parasitoids to develop themselves in their lepidopteran hosts. PDV genes play an important role in this process, by suppressing the immune system and altering feeding behavior, leading to the death of the lepidopteran host. The virulence genes, proteins and venom involved in the parasitization process represent a source of tools for pest control. Here, we have reviewed three methods using PDVs for pest control.

5.1 Parasitoids Used in the Biological Control

Since the interaction of parasitoids with their hosts using PDVs has been well-studied, parasitoids are widely used in biological control against crop pests (Clarke et al. 2019). They have been used as introduced biocontrol agents in classical biological control programs (Brewer et al. 2005). Parasitoids usually have a range of hosts and rarely only attack one host species (Barahoei et al. 2013, 2014). As an example, *Binodoxys communis* (Gahan) can attack several closely related aphid species (Raymond et al. 2016). The host range of parasitoids is not always the same in an entire species, as different races of parasitoids may be specific to different host species. Within the host rage, parasitoids have preferred hosts (Zepeda-Paulo et al. 2013; Henry et al. 2008). The first successful example of classical biological control used the predatory beetle *Rodolia cardinalis* (Mulsant) and the parasitic fly *Chryptochaetum iceryae* (Williston) to control cottony cushion scale, *Icerya purchasi Maskall* in California in 1888 (Heimpel and Cock 2018; Caltagirone and Doutt 1989). Since then, many successful classical biological control programs have been performed, such as the control of cassava mealybug (*Phenococcus manihoti*) by encyrtid parasitoids (*Epidinocarsis lopezi*) in sub-Sharan Africa and control of the diamondblack moth (*Plutella xylostella*) by the introduction of a parasitoid complex (Furlong and Zalucki 2017; Furlong et al. 2013; Alene et al. 2006). Biological control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid *Encarsia formosa Gahan* (Hymenoptera: Aphelinidae) was established in the UK in the 1920s and stopped with the introduction of pesticides in 1940. In the 1970s, this practice was re-introduced to control whitefly due to increasing problems with insecticide resistance and the environmental and health effects of pesticides (De Clercq et al. 2011). Another most studied example of using parasitoids for biological control is *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), an endoparasitoid wasp with PDV, which has been used to against *Busseola fusca*, a significant pest of maize and sorghum (Gundersen-Rindal et al. 2013).

To successfully implement biological control, studying parasitoid diversity and understanding intra- and interspecific plasticity is necessary. Parasitoids complete development in other insects, leading to their death or sterility; this offers an

excellent mechanism for natural and sustainable pest control (Clarke et al. 2019). For classical and enhanced biological control, the positioning of target pests depends on the interaction between parasites, target pests and crops. Parasitoids can be trained to become more efficient at different stages of the host search and host acceptance process; using this strategy, the regulation of parasite olfaction can lead to "foraging efficacy gain" (Kruidhof et al. 2019). Parasitoids recognize host and non-host species using chemical compounds to locate and accept their hosts. Enzymes in host oral secretions play a key role in host acceptance and oviposition by parasitoids (Clarke et al. 2019).

The use of biological control has huge economic and ecological benefits, such as the reduction of damage by agricultural and forestry pests, protection of biodiversity in the natural system and providing valuable ecosystem services (De Clercq et al. 2011). However, biological control agents also incur potential risks to the environment, including changes in the abundance or distribution of native species, transferring harmful pathogens to native species, biodiversity loss, genetic dilution of native species and so on (De Clercq et al. 2011).

5.2 PDV Genes Expressed in the Baculovirus Expression Vector System

Baculoviruses are used as bio-insecticides in biological control programs against lepidopteran pests, which have a narrow host range. They are harmless to non-target organisms, are highly pathogenic and stable in the environment (Szewczyk et al. 2006). Several wild-type baculoviruses are used for pest control, such as *Helicoverpa armigera nucleopolyhedrovirus* (HaMNPV) used on cotton, *Spodoptera frugiperda* NPV (SfMNPV) on maize and *Anticarsia gemmatalis* NPV (AgMNPV) on soybean (Moscardi 1999; Srinivasa et al. 2008). However, the application of the baculoviruses has limits due to its low median lethal time. Genetically engineered baculoviruses have been developed to enhance the insecticidal activity of wild-type baculoviruses. Some PDV genes significantly enhance baculovirus protein expression and kill more efficiently than wild-type viruses.

Five members of the CsIV cys-motif gene family have been produced using the baculovirus expression system, and the recombinant proteins were injected or fed to *H. virescens* in the diet. rVHv1.1 caused a significant reduction in the growth of *H. virescens* and *S. exigua* larvae. This protein also caused delayed development, reductions in pupation and increased mortality (Fath-Goodin et al. 2006). The cys-motif expressed by the recombinant baculovirus system affected lepidopteran physiology in terms of both immunity and development (Gundersen-Rindal et al. 2013).

In our lab, we constructed one recombinant baculovirus with the PDV gene CrV1. The CrV1 secreted protein of CrBV is responsible for actin depolymerization in hemocytes and the suppression of immune functions such as phagocytosis and cell

spreading, thus allowing the successful embryonic development of the parasitoid wasp. The recombinant baculovirus was tested against the insect pests *S. exigua* and *P. rapae*. The recombinant virus expressing CrV1 protein showed significantly lower lethal concentration 50 and lethal time 50 as compared with the wild-type virus, which indicated that recombinant baculoviruses expressing only the CrV1 gene have improved virulence (Wei et al. 2016a, b).

Another novel recombinant baculovirus, NeuroBactrus, was also constructed to use for biological control. In this construction, the *Bacillus thuringiensis* crystal protein gene (here termed cry1-5) and an insect-specific neurotoxin gene, AaIT, from *Androctonus australis* were introduced into the AcMNPV genome under different promoters. NeuroBactrus showed high insecticidal activity against *Plutella xylostella* larvae and a significant reduction in the median lethal time against *S. exigua* larvae compared to those of wild-type AcMNPV (Shim et al. 2013).

Two enhancing factors from *Agrotis segetum* granulovirus and *Cydia pomonella* granulovirus were constructed into AcMNPV. The recombinant viruses were tested against the second and fourth instars of *S. exigua* larvae, and the recombinant viruses showed four- to sevenfold lower median lethal doses compared to those of the wild-type virus. Further bioassays showed that the recombinant viruses were incapable of infecting the second instar larvae of *Spodoptera litura*, *H. armigera* and *Pyrausta nubilalis*, which were not sensitive to wild-type AcMNPV (Lei et al. 2020).

As described, PDV genes combined with baculovirus could be a good strategy for effective and environmentally friendly pesticide development.

5.3 Transgenic Plants Expressing PDV Genes

For insect control, transgenic approaches have also been used, by introducing insecticidal genes into plants, such as resistance genes, lectin genes, inhibitors of digestive enzymes and *Bacillus thuringiensis* (Bt) toxins (Zhao et al. 2003; Cavalieri et al. 1996; Dowd et al. 1998; Huesing et al. 1991). The first development of transgenic plants expressing a parasitoid gene was done in 2003, which introduced a single teratocyte secretory protein (TSP) 14 into tobacco. Teratocytes suppress host insect growth and development and cause immunosuppressive conditions in the host (Dahlman and Vinson 1993). Teratocytes produce TPSs in host hemolymph, and this protein expressed in vitro injected into host larvae produced similar responses to parasitization (Schepers et al. 1998). TSP14 has a cysteine-rich motif and shares significant sequence similarity with proteins of the Cys-motif gene family, which are encoded by the ichnovirus associated with CsIV. The transgenic plants were tested against *H. virescens*. The results showed that the growth and/or development in *H. virescens*, as well as plant damage, were significantly reduced and the mortality rates were higher compared with controls (Maiti et al. 2003).

Another viral gene from CpBV, CpBV-CST1, was also introduced into tobacco. Bioassay results showed that young larvae of *S. exigua* exhibited high mortality after

feeding on transgenic tobacco, which suggests the prospective possibility of using PDV genes in a transgenic approach to pest control (Kim et al. 2016).

A virulence factor encoded by *Toxineuron nigriceps* bracovirus (TnBV), a member of the Ank protein family, TnBVANK1, was genetically modified into tobacco plants. This gene is involved in both immunosuppression and endocrine alterations in the host. Transgenic tobacco plants showed insecticidal activity and caused developmental delay in *Spodoptera littoralis* larvae feeding on them (Di Lelio et al. 2014).

Transgenic plants with parasitoid-derived genes open a new door to insect control. Future work must be done to evaluate any effect on non-target organisms, the environment and humans.

6 Conclusion

Regarding PDV, many questions still need to be answered, such as the ancestor of IVs and the mechanism of PDV gene interactions with the immune defenses of the host. Further scientific research is encouraged to answer these questions. With the further development of sequencing technology, more species of PDV and/or more PDV genes involved in the parasitization process will be identified. PDV genes are a rich source of viral genes that could be applied to pest control. Many approaches to using PDV genes could be further studied for novel control methods against pest insect species.

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