Polydnaviruses: Nature's Genetic Engineers

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Abstract

Virus-host associations are usually viewed as parasitic, but several studies in recent years have reported examples of viruses that benefit host organisms. The Polydnaviridae are of particular interest because these viruses are all obligate mutualists of insects called parasitoid wasps. Parasitoids develop during their immature stages by feeding inside the body of other insects, which serve as their hosts. Polydnaviruses are vertically transmitted as proviruses through the germ line of wasps but also function as gene delivery vectors that wasps rely upon to genetically manipulate the hosts they parasitize. Here we review the evolutionary origin of polydnaviruses, the organization and function of their genomes, and some of their roles in parasitism.

INTRODUCTION

Parasitoid: insect with a parasitic larval stage that develops by feeding on one host; most are Hymenoptera

Hymenoptera: a major order of insects with more than 200,000 species; many are ants or bees, but most (70–80%) are parasitoids

Among the many biological advances of the nineteenth century, two of the more important were the concept of symbiosis as defined by Heinrich Anton de Bary (1) and the discovery of the first virus by Martinus Beijerinck (2). Symbiosis, the living together of dissimilar organisms, encompasses interactions ranging from beneficial (mutualism) to neutral (commensalism) to deleterious (parasitism). Contemporary microbiologists recognize that bacteria, protozoans, and fungi have evolved a full spectrum of symbiotic associations with other organisms, which has contributed greatly to the generation of biological diversity (3–5). Virologists, in contrast, have primarily focused on disease-causing species, which has led to the view that virus-host associations are primarily parasitic. Reinforcing this perspective is the etymology of the term virus, meaning "poison" in Latin, and most definitions, which refer to viruses as intracellular parasites because they require a living host cell to reproduce (i.e., they cannot self-replicate) (6, 7). However, when viewed in terms of de Bary's definition of symbiosis, the broader literature reveals that some viruses persist without causing any apparent deleterious effects in hosts, others are facultative mutualists, and a few are obligate mutualists in which neither virus nor host can survive without the other (8–17).

The best example of the latter is the family Polydnaviridae, which are large, double-stranded DNA (dsDNA) viruses associated with insects called parasitoid wasps (Hymenoptera). Polydnaviruses (PDVs) are also of interest because their genomes are organized and function in ways that differ from any other virus group (17). Several recent summaries describe different aspects of PDV biology (17–24). Here, we review the current state of knowledge on PDV evolution and function.

THE POLYDNAVIRUS-PARASITOID MUTUALISM

Parasitoid wasps are free-living insects as adults that reproduce by laying eggs on or into the bodies of other arthropods (hosts) their progeny consume (25, 26). Parasitism almost always results in the death of the host. Most wasp species have also specialized to parasitize only one or a few host species, which is often associated with high mortality of host populations (27). One consequence of these life history traits is that parasitoid wasps and hosts experience coevolutionary arms race interactions in which selection favors traits in wasps that facilitate parasitism of particular hosts and counteradaptations in hosts to resist parasitism by particular wasps. Another is that parasitoid wasps have diversified into one of the most species-rich animal groups on Earth, with estimates suggesting 20–25% of all insects are parasitoids (25, 28). It is in this context that PDVs evolved.

Polydnaviruses Are Associated with Two Families of Parasitoid Wasps

PDV particles were first observed about 40 years ago (29–32), and the Polydnaviridae was recognized as a family by the International Committee on Taxonomy of Viruses in 1991 (33). All PDV-carrying wasps belong to a superfamily named Ichneumonoidea, which consists of two families, the Braconidae and Ichneumonidae, that diverged approximately 150 million years ago (**Figure 1**) (34, 35). The Polydnaviridae is also currently divided into two genera named the *Bracovirus* and *Ichnovirus*. Bracoviruses (BVs) are associated with an estimated 50,000 species of braconids in six subfamilies that form a monophyletic assemblage called the microgastroid complex (36, 37). The microgastroid complex diverged approximately 100 million years ago from other subfamilies of braconids that lack BVs (34, 37). Ichnoviruses (IVs) are associated with approximately 14,000 species of ichneumonids in two subfamilies. The phylogenetic relationship of these subfamilies to one another remains uncertain, and approximately 23 other subfamilies of ichneumonids lack IVs (20, 38).

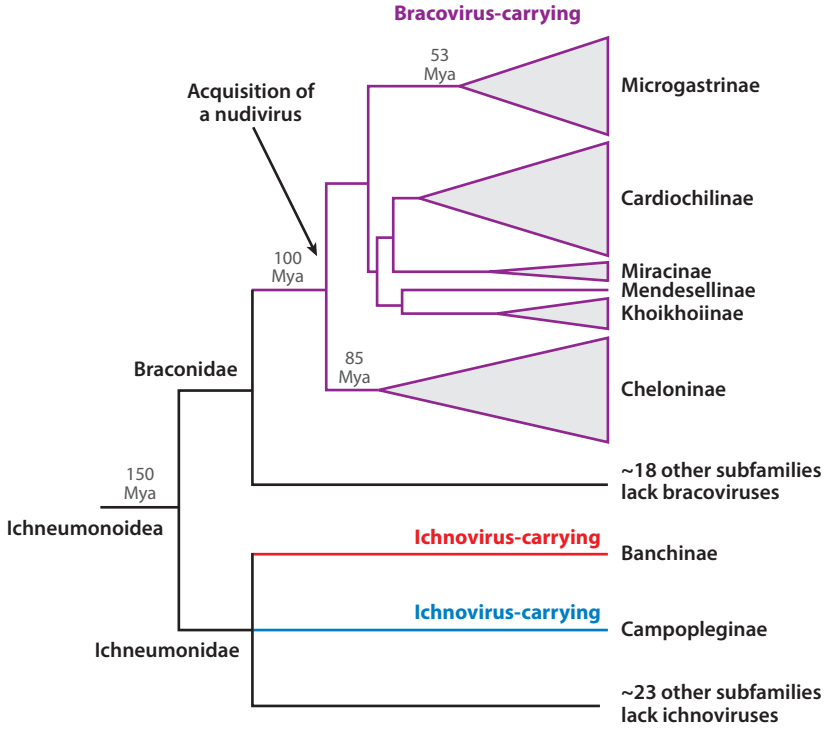


Figure 1

Evolutionary relationships of polydnavirus-carrying wasps in the superfamily Ichneumonoidea. The microgastroid complex (Braconidae) contains six subfamilies that carry bracoviruses (*purple*). The terminal triangles indicate the relative abundance of wasp species in each of these subfamilies. The estimated acquisition date of the nudivirus ancestor of bracoviruses and divergence times of select subfamilies are indicated in millions of years ago (Mya) as inferred from fossil records and other data (34–38). Other branches show braconid subfamilies that lack bracoviruses and subfamilies of Ichneumonidae, including Banchinae (*red*) and Campopleginae (*blue*), that carry ichnoviruses.

The Polydnavirus Life Cycle

Each PDV persists as an integrated provirus in the germ line and somatic cells of every individual of a given wasp species. As such, PDVs are endogenous virus elements (EVEs) that have become genetically fixed in different wasp lineages (13, 22, 24, 39, 40). Unlike other known EVEs of ancient origin, however, PDVs retain the ability to replicate, which occurs only in female wasps in the nuclei of calyx cells that are located in the reproductive system (**Figure 2*a***). Replication begins during the mid-pupal phase and usually continues during adulthood (41–46). The virions that assemble in calyx cells contain multiple circular dsDNAs that are nonequimolar in abundance and have large aggregate sizes (190–730 kb). The name Polydnaviridae derives from this feature, and the totality of DNA segments packaged into virions during replication is referred to as the encapsidated form of the genome (17, 18). Once assembled, virions are released from calyx cells and stored in the lumen of the oviducts with mature wasp eggs (**Figure 2*a***) (47).

Endogenous virus element (EVE): a DNA sequence of virus origin present in the germ line of a nonviral organism

The hosts of PDV-carrying wasps are primarily members of the order Lepidoptera (moths and butterflies) that females parasitize during the larval stage or, in the case of chelonine braconids, as eggs (27, 48). Wasps use their flexible ovipositor to inject into the body of the host one or more eggs containing the proviral genome, PDV virions, and secretions from other organs such

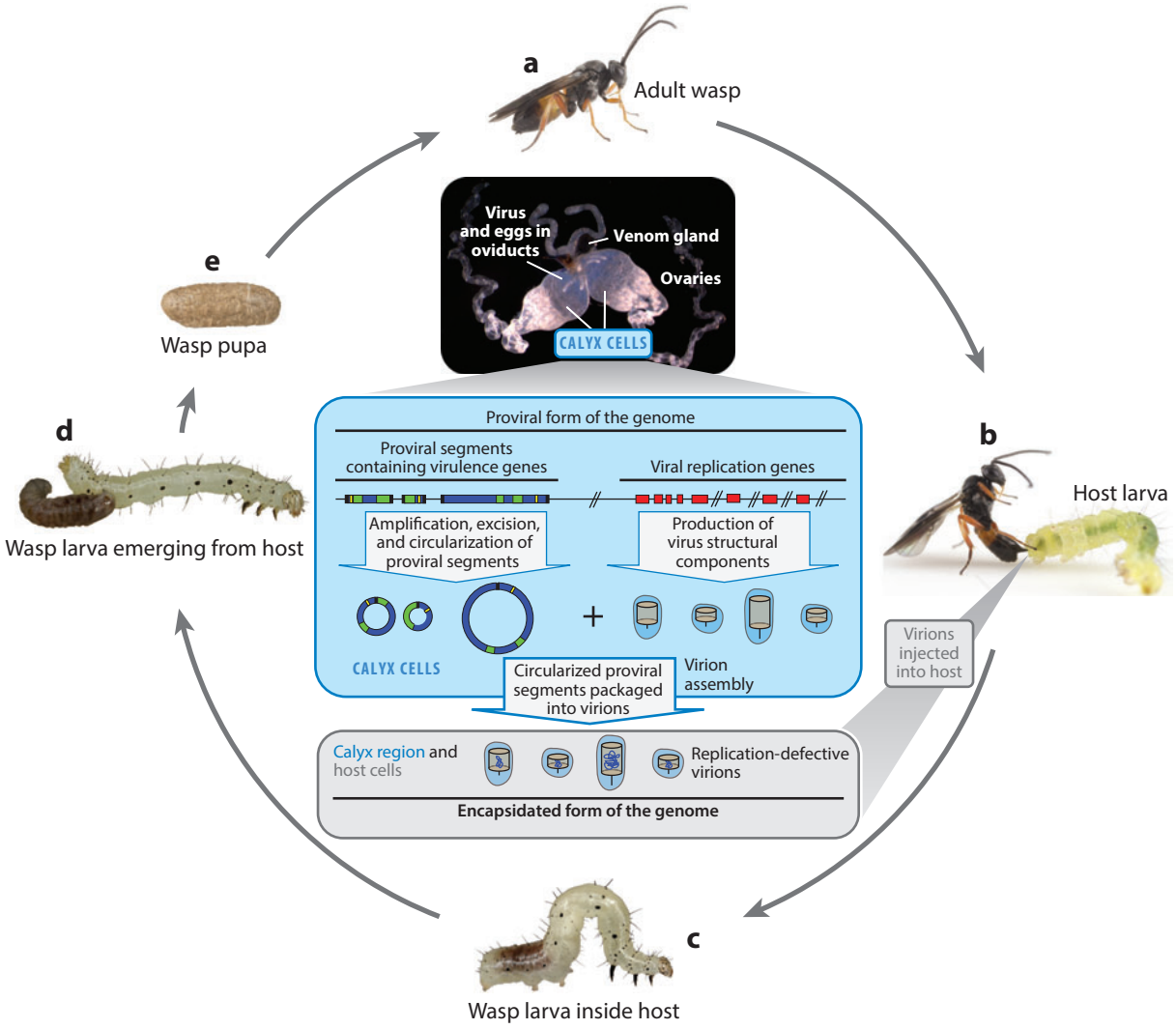


Figure 2

Polydnavirus life cycle and genome organization as understood from the study of bracoviruses. (*a*) Adult female wasp. The reproductive system (*below*) includes two ovaries and a venom gland. Calyx cells reside between the ovaries and oviducts. Replication in calyx cells involves two components of the proviral genome: expression of genes required for virion formation (*red*) and amplification, and circularization of DNAs containing virulence genes that are packaged into virions (*blue*). Wasp eggs and virions are stored in the lateral oviducts of the wasp. High densities of virus particles in the ovaries confer a blue color. (*b*) Parasitism of a host larva by the adult wasp. The wasp injects eggs, virions containing the encapsidated form of the genome, and venom gland secretions. (*c*) Developing wasp larva inside the body of the host. (*d*) Wasp larva emerging from the host to pupate. The host dies several hours or days after emergence of the wasp. (*e*) Wasp pupa. In females, virus replication begins in the mid-pupal stage.

as the venom gland (**Figure 2*b***). Virions infect host cells by discharging their DNAs into the nuclei, which is followed by expression of viral genes and integration of DNA segments into the genome of infected host cells (17, 20, 47). These PDV gene products have two main functions: (*a*) They immunocompromise the host, which prevents wasp offspring from being killed, and (*b*) they alter host growth, which promotes wasp offspring development and often causes the host

to die (**Figure 2*c*,*d***) (50, 51). Upon emergence from the pupal stage (**Figure 2*e***), adult males and females mate, after which females forage for new hosts to parasitize.

On first inspection, PDVs do not seem unique, because several viruses infect more than one host and/or cycle between persistence as proviruses and replication to produce virions that transmit the genome to new hosts. What distinguishes PDVs is that their proviral genomes have evolved into two functional units: (*a*) a suite of genes with replication functions that produce virions in calyx cells and (*b*) multiple DNA domains containing virulence genes that are amplified and packaged into virions (**Figure 2**). Remarkably, replication genes are transcribed in calyx cells, but none reside in the packaged DNA domains (21–24). Reciprocally, almost none of the genes on packaged DNAs are transcribed in wasps, but all are transcribed in the hosts wasps parasitize (52). In all other wasp cells the proviral genome is inactive, whereas in parasitized hosts PDVs cannot replicate. PDVs thus function as gene delivery vectors wasps use to genetically manipulate host insects. PDVs and wasps are also obligate mutualists, because each PDV can only be transmitted vertically through the germ line of its wasp and each wasp relies on its PDV for survival in hosts.

POLYDNAVIRUS EVOLUTION

The unique biology of PDVs raises many questions. Perhaps the most important is, what did they evolve from? Although it is counterintuitive given their shared life cycle, early studies noted that BVs and IVs might have different origins because their virions are dissimilar (53, 54). BV virions assemble de novo in calyx cell nuclei and consist of cylindrical nucleocapsids with tails that are surrounded by a single unit membrane (32, 55). BVs also package only one DNA segment per virion, which is followed by release of virions via calyx cell lysis (41, 56). In contrast, IVs produce virions consisting of fusiform nucleocapsids and an envelope that may package multiple DNAs (18, 47). IVs then exit calyx cells by budding through the plasma membrane, which results in acquisition of a second envelope (47, 57). Morphological differences have also been noted between banchine and campoplegine IVs, which raises the possibility they, too, have different origins (58, 59). Further supporting an independent origin for BVs and IVs are the monophyly of BV-carrying braconids and the large phylogenetic distance between these wasps and the subfamilies of ichneumonids with IVs (**Figure 1**). The former indicates BVs evolved from a single ancient ancestor, whereas the latter suggests independent acquisition of BVs and IVs is more likely than loss of the association in the numerous other subfamilies that lack PDVs.

The Encapsidated Genomes of Polydnaviruses Provide No Insights About Ancestry

Genome analysis is obviously critical to addressing ancestry, but understanding the origin(s) of PDVs was stymied initially by sequence data because workers in the field, us included, thought like traditional virologists initially and focused on the encapsidated form of PDV genomes. Five BV genomes [*Cotesia congregata* BV (CcBV) (60), *Cotesia plutellae* BV (CpBV) (61), *Microplitis demolitor* BV (MdBV) (62), *Glyptapanteles indiensis* BV (GiBV), and *Glyptapanteles flavicoxis* BV (GfBV) (63)], three IV genomes from campoplegine ichneumonids [*Campoletis sonorensis* IV (CsIV) (62), *Hyposoter fugitivus* IV (HfIV) (64), and *Tranosema rostrale* IV (TrIV) (64)], and one IV genome from a banchine ichneumonid [*Glypta fumiferanae* IV (GfIV) (58)] have been fully sequenced. Several other BV and IV isolates have also been partially sequenced (summarized in 21, 23).

Besides segmentation, these data identify other shared organizational features, including (*a*) low gene-coding densities; (*b*) a mix of genes with and without introns; (*c*) several multimember gene families; and (*d*) a number of genes with homology to known genes from wasps, other insects, or other eukaryotes. They also show BVs from closely related wasps share several genes with one

another, as do closely related IVs, whereas more distantly related BVs or IVs share fewer genes (20, 23, 65–67). However, BVs and IVs share almost no genes with one another, and neither group contains any homologs of viral genes with functions in replication. Taken together, the encapsidated genomes of BVs and IVs show concordance with braconid and ichneumonid phylogeny (34, 37, 63, 64, 68, 69), which is a pattern seen in many vertically inherited obligate endosymbionts (3, 70). The paucity of shared genes between BVs and IVs also supports their independent origin while suggesting their shared features reflect convergent evolution driven by analogous roles in parasitism. In contrast, the insect-like architecture of PDV encapsidated genomes and the absence of replication genes initially argued against a distinct virus ancestor for either (71).

Core genes: genes in a given virus family for which orthologs have been identified in all sequenced species

Baculovirus DNA-dependent RNA polymerase: composed of four subunits that recognize a unique promoter consensus sequence present in structural and other late or very late genes

***vlf-1* gene:** very late factor identified from baculoviruses as encoding a structural component of nucleocapsids; based on sequence, it is an integrase family member

***pif* genes:** first identified from baculoviruses as per os infectivity factors required for infection of insects; encode envelope components of occluded virions

The Ancestor of Bracoviruses Was a Nudivirus

The first insights that PDVs are of viral origin came from transcriptome studies in three braconid wasps (*Cotesia congregata*, *Chelonus inanitus*, and *Microplitis demolitor*), which identify 42 viral genes expressed in ovaries during replication (46, 68, 72). All of these genes share weak but recognizable homology with genes from another group of insect-infecting DNA viruses called nudiviruses. Nudiviruses are poorly studied functionally, but comparative genomic data indicate they are the sister taxon to Baculoviridae, for which replication of model species such as *Autographa californica* multinucleopolyhedrosis virus (AcMNPV) has been extensively characterized (**Figure 3**) (73, 74). Baculoviruses and nudiviruses diverged approximately 300 million years ago (75). Most baculoviruses and many nudiviruses are virulent pathogens of insects that establish systemic, fatal infections by undergoing lytic replication in all cells of an infected host and expressing different virulence genes (73). However, two nudiviruses have been identified that infect the reproductive system of insects and in vitro establish persistent infections associated with integration into the host genome (76, 77). Such latent infections can also reactivate to reestablish lytic infections (77, 78).

Like other large DNA viruses, baculoviruses exhibit high diversity in gene content, but all are thought to share 37 core genes, of which about half are required for replication (73, 79). These include a DNA polymerase (*dnapol*) that replicates the viral genome, four subunits of a novel DNA-dependent RNA polymerase, and several structural genes with promoter sequences specifically recognized by the viral RNA polymerase (**Figure 3**). Six nudivirus genomes have been sequenced, and each encodes 20 baculovirus core gene homologs including *dnapol*; RNA polymerase subunits (*lef-4*, *lef-8*, *lef-9*, and *p47*); the initiation factor *lef-5*; and several structural genes, including *vp39*, *vlf-1*, *p74*, and multiple *pif* genes (**Figure 3**) (80). The functions of these genes, however, are unknown beyond inferences from baculoviruses.

Nudivirus-like genes upregulated in calyx cells include the four RNA polymerase subunits and the above-mentioned structural genes, which in some cases have diversified into multigene families (**Figure 3**). Other predicted members of a BV conserved gene set are multiple variants of a tyrosine recombinase gene named *integrase*, which is unknown from baculoviruses but is related to *vlf-1* (**Figure 3**). In contrast, no homologs of baculovirus or nudivirus genes involved in viral DNA replication have been identified except for a *helicase* in *M. demolitor* (46). These data, together with the monophyly of microgastroid braconids, strongly indicate that BVs evolved approximately 100 million years ago from a nudivirus.

The Ancestor(s) of Ichnoviruses Remains Unknown

Similar studies of IV-carrying campoplegine ichneumonids identify no nudivirus or baculovirus genes with roles in replication (81). However, domains in the genomes of three wasps (*Hyposoter*

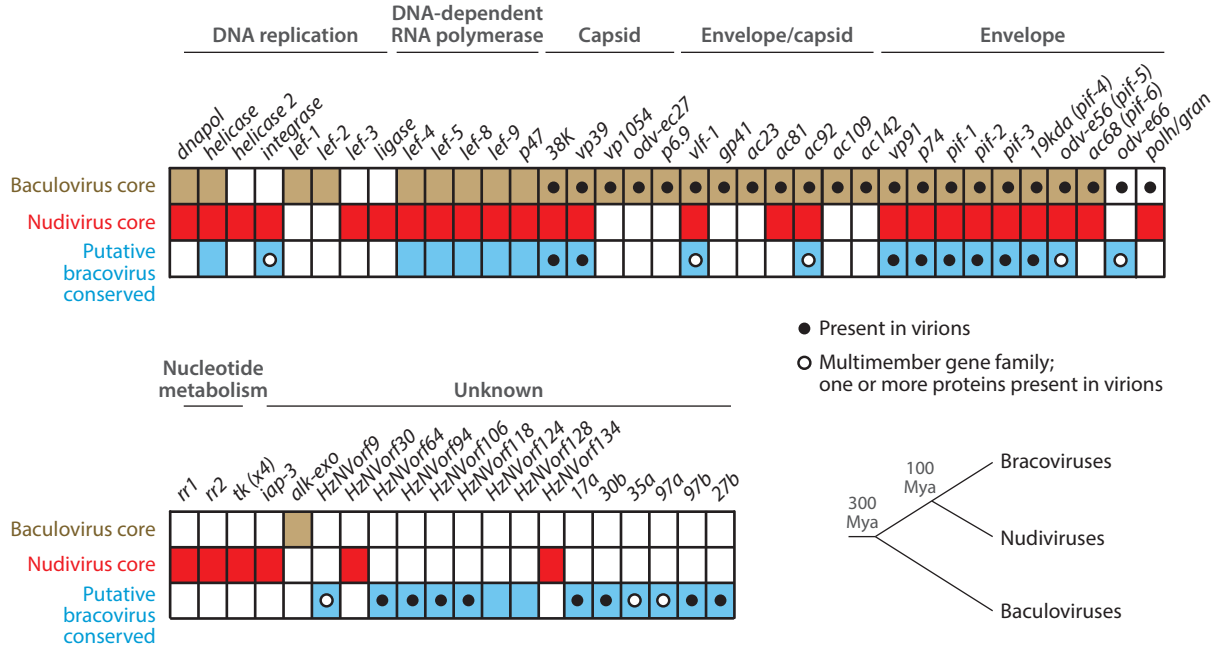


Figure 3

Core gene content of baculovirus and nudivirus genomes and their conservation in bracovirus proviral genomes. Columns show genes organized by their experimentally determined functions in baculoviruses. Colored boxes indicate the presence of a gene in all sequenced baculovirus genomes (*tan*), nudivirus genomes (*red*), or bracovirus proviral genomes (*blue*). Gene products present in baculovirus and bracovirus virions are highlighted with filled circles. Open circles indicate that one or more members of a multigene family are detected in bracovirus virions. A cladogram (*lower right*) shows evolutionary relationships for each virus group, with ages at nodes indicated in millions of years ago (Mya). Age estimates based on insect fossil records calibrate the divergence between nudiviruses and bracoviruses (75).

didymator, *Campoletis sonorensis*, and *Tranosema rostrale*) contain genes for structural proteins in IV virions (81, 82). These IV structural protein–encoding regions (IVSPERs) share no homology with genes from any known virus. Thus, IVSPERs have organizational features suggesting IVs evolved from a virus that integrated into the germ line of an ichneumonid ancestor. Whether this ancestor belongs to a now-extinct or undiscovered virus group, however, is unknown. Also unknown is whether the IVs in banchine ichneumonids have IVSPERs that are similar to or different from those in campoplegine ichneumonids.

BRACOVIRUS GENOME ORGANIZATION AND FUNCTION IN WASPS

Because some nudiviruses infect the reproductive system of insects and establish latent infections, a plausible scenario for BV evolution is that their ancestor established a latent infection in the reproductive tract of a braconid wasp (68). Given the diversity of arthropods infected by nudiviruses (80), this could have occurred by direct infection of the ancestral wasp or horizontal acquisition from a host of the ancestor wasp. Selection then favored alterations that resulted in the mutualism that exists today. Recent efforts have focused on understanding what these alterations are and how they affect BV function in wasps. In contrast, little is yet known about IV function in wasps because much of their proviral genome(s) remains unidentified.

Nudivirus-Like Genes Retain Ancestral Functions

Integrases: a diverse family of tyrosine recombinases, which rearrange DNA duplexes by means of conservative site-specific recombination reactions

Helicases: enzymes that separate strands of a DNA double helix, an activity required in many processes, including DNA replication

The first nudivirus-like genes expressed at the onset of replication are the RNA polymerase subunits and integrases; this is followed by the transcription of structural genes, which coincides with amplification and circularization of proviral segments packaged into virions (46, 68, 83). These patterns clearly suggest a role for the baculovirus/nudivirus-like genes in virion formation. However, algorithms such as BLAST cannot detect homology between BV and baculovirus core genes, whereas identity with nudivirus homologs ranges from 19% to 41% (46). Such patterns are not surprising given that BVs and nudiviruses diverged 100 million years ago and the last common ancestor of BVs, nudiviruses, and baculoviruses existed 300 million years ago (75). However, these data also raise the question of whether BV nudivirus-like genes retain ancestral functions.

Two lines of study indicate they do. First, proteomic analysis of BV virions from *C. congregata*, *C. inanitus*, and *M. demolitor* show that products corresponding to all of the baculovirus-like capsid and envelope genes are present (**Figure 3**) (72, 83). No proteins corresponding to *helicase* or RNA polymerase subunits are detected in virions. However, proteins corresponding to *int-1*, *vlf-1b-1*, and *vlf-1b-2* are detected, which is notable given that VLF-1 is a baculovirus capsid protein and BV DNAs integrate into the genome of cells from host insects (84–86). Second, experiments in *M. demolitor* using RNA interference show that (*a*) the nudivirus-like RNA polymerase subunits produce a functional enzyme that transcribes structural genes but not wasp genes; (*b*) *vp39*, *p74*, and *pif-1* are required for virion formation; and (*c*) *vlf-1* and *int-1* exhibit recombinase functions required for excision and circularization of proviral DNA segments (83). Thus, the structural genes BVs use to produce virions exhibit conserved functions with baculoviruses, whereas the integrases exhibit functions that are unknown from baculoviruses but may be important in nudiviruses that establish latent infections. In contrast, the absence of a baculovirus-like *dnapol* and most other genes required for baculovirus genome replication suggests amplification of the DNA segments packaged into virions is regulated by wasp machinery.

Bracovirus Proviral Genomes Are Dispersed

Nudiviruses and baculoviruses package a single large circular dsDNA genome (>100 kb) into virions (73, 74, 80). Integration of the nudivirus ancestor into the germ line of the wasp ancestor of BVs thus presumably resulted in a linear DNA with the resulting provirus initially retaining the ability to produce virions capable of replicating. In contrast, sequencing of bacterial artificial chromosome clones from genomic libraries of four braconid species (*Cotesia congregata*, *Cotesia sesamiae*, *Glyptapanteles indiensis*, and *Glyptapanteles flavicoxis*) along with recent whole-genome sequencing of *M. demolitor* indicates BV proviral genomes are dispersed (63, 68, 87, 88).

Consider first the nudivirus-like genes, which are all intronless. In *M. demolitor*, 25 of these genes, including several virion components such as *vp39*, *38K*, and *pif-3*, reside in a 75-kb cluster flanked by wasp genes with introns (**Figure 4*a***). A subset of these genes is present in the same order and orientation in the *C. congregata* genome (68), which indicates a number of nudivirus-like genes have remained stable in braconid genomes since the divergence of the genera *Cotesia* and *Microplitis* 53 million years ago (37). Other nudivirus-like genes, including *helicase*, *lef-9*, *vlf-1*, and *p74*, are located singly on different scaffolds, which indicates they are widely dispersed.

Consider next the DNA segments packaged into virions. Sequencing of these DNAs from virus particles had already shown that, unlike the conserved nudivirus-like genes, they vary with wasp phylogeny; as a result, homologous segments are recognizable between BVs associated with wasp species from the same or closely related genera, but little or no recognizable homology is discernible between BVs associated with wasps in different subfamilies or distantly related genera

(63, 66). In contrast, sequencing in their proviral form reveals three features not apparent from examining only the circularized segments (**Figure 4*b***). The first is how segments are organized. In *G. indiensis* and *G. flavicoxis*, six loci containing segments have been identified (63). Loci 1 and 2 form a "macrolocus" of 13 and 8 tandemly arrayed segments linked by <100 kb of DNA containing wasp genes, and loci 3 through 6 consist of 1 or 2 segments (63). In *C. congregata* and *C. sesamiae*, 9 loci with similar architecture are present (87), whereas in *M. demolitor*, proviral segments reside in 8 loci on 11 scaffolds that also share similar architecture (88). For some segments, surrounding genomic regions are homologous between wasp species, implying common ancestry, even though relatedness of the proviral segments themselves has been obscured by rapid evolution. The distribution of DNA segments in *M. demolitor* also strongly suggests proviral segment loci are not clustered in one region of the wasp genome.

ODV-E66: an envelope protein first identified in occluded virions from baculoviruses

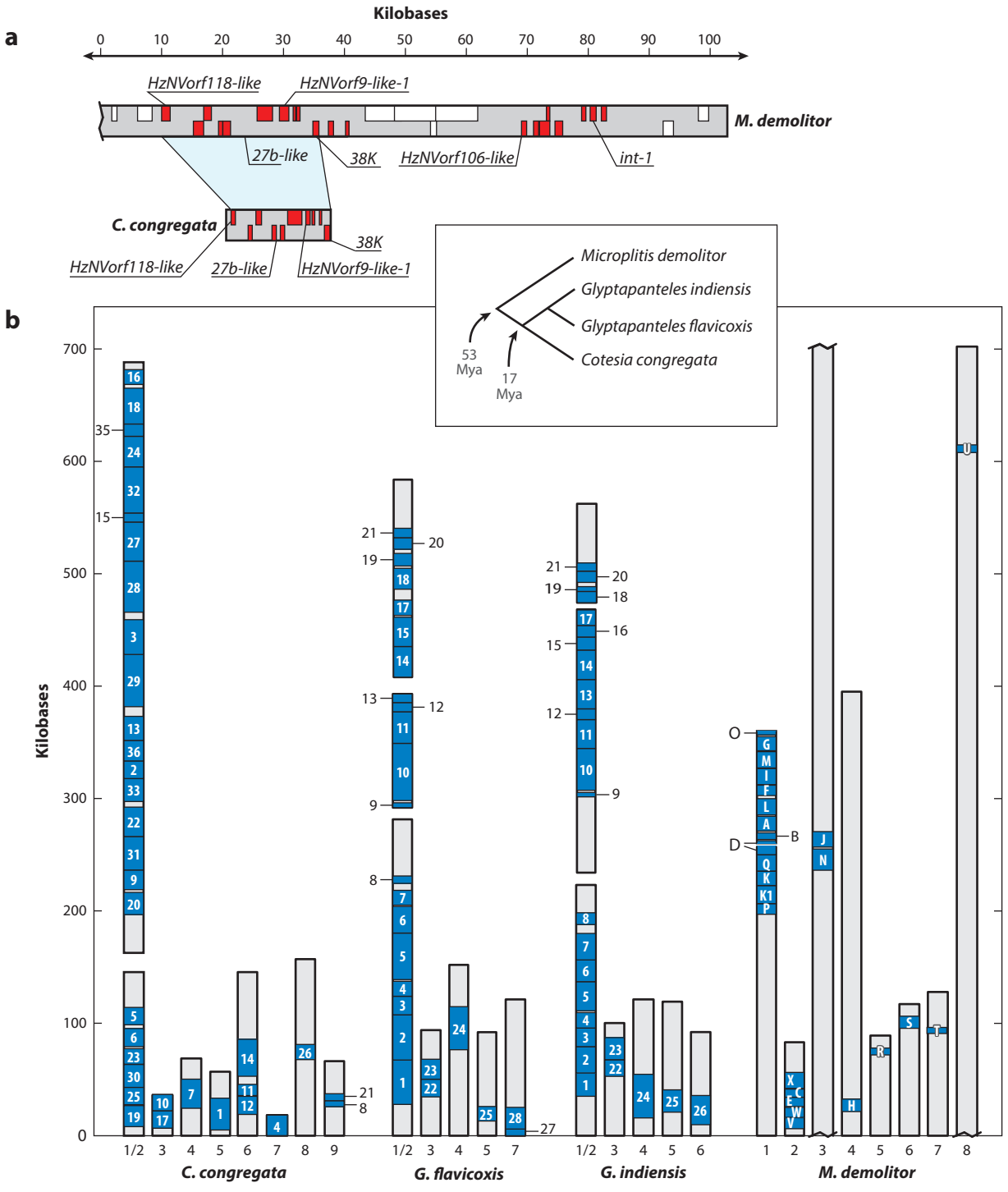
A second feature is that BV proviral segments share similar flanking junctions containing the tetramer AGCT (84, 87, 89, 90). These direct repeats, also called wasp integration motifs (WIMs) (84), identify the site of proviral segment circularization that occurs during replication. None of the nudivirus-like genes have WIMs, but the dependence of proviral segment processing and circularization on *vlf-1* and *int-1* in *M. demolitor* suggests WIMs derive from the ancestral nudivirus genome (83). The third feature is that the WIM in circularized segments plays no role in the reintegration of circularized segments into the genome of parasitized hosts, which instead occurs at another domain named the host integration motif (HIM) (84). HIMs are 100–110-bp imperfect, inverted repeats that form a stem-loop structure, with integration into the host genome resulting in deletion of the predicted loop (84). HIMs are also present in proviral segments from *Cotesia* and *Glyptapanteles* BVs (24).

The location of the nudivirus-like genes and proviral domains relative to one another remains unclear. One structural gene, *odv-e66-like 1*, is present between loci 1 and 2 of *C. congregata*, which provides a second piece of evidence that the nudivirus-like machinery required for virion formation and the DNA segments packaged into virions have a common origin (24). No *odv-e66* gene resides in this region in *M. demolitor*, but other genes and motifs exist that also support a common origin for the nudivirus-like genes and proviral segments. Other data suggest the MdBV proviral genome is widely dispersed in *M. demolitor*. How did dispersal occur? One possibility is that the ancestral nudivirus genome duplicated after integration; another is that several copies of the genome initially integrated, followed by elimination of conserved replication genes from some and elimination of WIMs from others (17, 23, 24).

Genome Dispersal Maintains Bracoviruses as Mutualists

A number of ancient EVEs from different viruses have been identified in animals, plants, and fungi (91, 92). Most are no longer viruses, because only fragments from a parental genome remain. Most are also nonfunctional due to mutation, with only a few examples known of single genes or regulatory domains with functions that hosts have exapted for new activities (13, 93, 94). In this light, BVs are extraordinary, because they consist of many genes that retain ancestral functions wasps use to genetically manipulate hosts (22).

How is this possible? Although virologists tend to think of viral genomes as contiguous stretches of DNA or RNA, the dispersed genomes of BVs clearly retain the ability to replicate. This is likely because the BV RNA polymerase holoenzyme, once made, transcribes all of the structural genes through promoter recognition regardless of their location in the wasp genome. In turn, WIMs serve as recognition motifs the nudivirus-like integrases use to process the DNAs packaged in virions. The reactions VLF-1 and INT-1 mediate are incompletely understood, although data for other tyrosine recombinases suggest four enzyme monomers form a synapse between their


a
Kilobases
0 10 20 30 40 50 60 70 80 90 100
HzNVorf118-like
HzNVorf9-like-1
M. demolitor
27b-like
38K
HzNVorf106-like
int-1
C. congregata
38K
HzNVorf118-like
27b-like
HzNVorf9-like-1
b
Microplitis demolitor
Glyptapanteles indiensis
Glyptapanteles flavicoxis
Cotesia congregata
53 Mya
17 Mya
Kilobases
0 100 200 300 400 500 600 700
C. congregata
G. flavicoxis
G. indiensis
M. demolitor

cognate binding sites to perform two strand exchanges that result in circularization (95, 96). What is clear, however, is the absence of WIMs around the genes required for BV replication results in none being packaged into virions.

MicroRNA: a small noncoding RNA molecule that functions in transcriptional or posttranscriptional regulation of gene expression

Dispersal of the proviral genome is therefore a key adaptation for producing replication-defective virions while assuring the whole BV genome remains with the wasp. Another potentially important adaptation in maintenance of BVs as mutualists is the loss of most ancestral viral DNA-replication genes, which suggests wasps control the amplification of DNAs packaged into virions (46). Finally, many large DNA viruses, including baculoviruses, have genomes containing many virulence genes of diverse origin that vary between species (73). That BV proviral genomes also contain many virulence genes is thus not unique. What is unique relative to the ancestor of BVs is that almost none of these genes are transcribed in wasps, even though all are transcribed in the hosts wasps parasitize (52). The molecular basis for this dichotomy remains unclear. However, its functional consequence—together with replication occurring only in calyx cells—is that BVs cause no disease in wasps.

Several questions pertaining to replication remain unanswered. For example, what activates replication in female wasps at the mid-pupal stage, and why is replication restricted to calyx cells when most baculoviruses and nudiviruses replicate in all cells of infected hosts? Part of the answer to these questions is that the BV RNA polymerase subunits and integrases are transcribed only in calyx cells, which, together with the wasp machinery that amplifies the proviral segments, are the key regulators of virion assembly (83). A wasp RNA polymerase presumably transcribes these genes, but the signal(s) that activate transcription are unknown. Candidates include hormones that regulate oogenesis in insects (97–99). However, data from *C. inanitus* show titers of these hormones do not correlate with the onset of replication, and experiments in *M. demolitor* indicate they do not activate replication (44; G.R. Burke & M.R. Strand, unpublished data). Factors implicated in latency of one nudivirus, HzNV-1, include the genes *hhi1* and *pag1*, which produce noncoding microRNAs (77, 78). MicroRNAs are well-known regulators of virus function in animals including insects (100, 101) and have also been implicated in regulating latency of herpesviruses (102, 103). However, no recognizable *pag1* or *hhi1* homologs have been identified in the genomes of BV-carrying wasps. Another unknown is the identity of the wasp genes that amplify the proviral segments and the mechanism of amplification. Transcriptome sequencing of *M. demolitor* indicates all standard insect DNA polymerases are expressed in ovaries and none exhibit expression patterns that correlate with segment amplification (46). Thus, transcriptional regulation of one or more wasp DNA polymerases likely does not control when amplification occurs. Baculovirus genomes are thought to replicate by a rolling circle or recombinational mechanism (73). In contrast, some

Figure 4

Organization of bracovirus proviral genomes based on sequencing of bacterial artificial chromosome clones from *Cotesia congregata*, *Glyptapanteles flavicoxis*, and *Glyptapanteles indiensis* and whole-genome sequencing of *Microplitis demolitor*. For each species, sections of the genome have been sequenced and assembled into large scaffolds. (*a*) Nudivirus-like gene clusters identified in *M. demolitor* and *C. congregata*. Nudivirus-like genes are indicated in red, with names indicated for a subset. Nonviral wasp genes are indicated in white. Regions between the two genomic scaffolds joined by light blue shading are syntenous. To economize space, other nudivirus-like genes identified in *M. demolitor* that reside on scaffolds outside this cluster are not shown. No data are currently available for nudivirus-like genes in *G. flavicoxis* and *G. indiensis*. (*b*) Organization of the proviral segments packaged into virions. Dark blue boxes depict proviral segments, which are identified numerically in *C. congregata*, *G. flavicoxis*, and *G. indiensis* (63, 87) and alphabetically in *M. demolitor* (62). Scaffolds are placed next to each other if they are known to originate from the same genomic region (i.e., locus) and are named numerically. The loci for the two species of *Glyptapanteles* are homologous (63), whereas the naming of loci in *C. congregata* and *M. demolitor* is partially homologous, with the scaffolds containing the largest number of proviral segments designated 1 and 2. The cladogram shows the evolutionary relationship between wasp species with estimated divergence dates in millions of years ago (Mya) (37).

data indicate BV DNAs amplify linearly, with segments in some loci coamplifying together before excision and circularization (44, 45, 104, 105).

Ankyrin repeat: a motif of approximately 33 amino acids implicated in regulating protein-protein interactions

Protein tyrosine phosphatases: enzymes that remove phosphate groups from phosphorylated tyrosine residues on proteins and regulate many signal transduction pathways

BRACOVIRUS FUNCTION IN THE HOSTS OF WASPS

Bracovirus Virions Rapidly Infect Host Cells

BV-carrying wasps inject sufficient virions into hosts to infect most immune cells (hemocytes) as well as many cells in other tissues (52, 56, 106, 107). BV entry into host cells is likely similar to that of baculoviruses given the conservation in virion architecture (84). Upon entry into a host cell, BVs traverse the cytoplasm and discharge their DNAs into the nucleus through nuclear pores; detectable levels of gene expression follow within 2 h of infection (47, 107). Early studies suggested BV DNAs persist as episomes (18, 47, 107, 108), but as previously noted, recent studies show a majority of the DNAs in host cells integrate (84). A potentially important advantage of integration is that many wasps overwinter (diapause) in their hosts, which requires that BV DNAs persist for long periods. Packaging of integrase proteins into virions further suggests BVs catalyze their own integration (83).

Variation in Proviral Segments Reflects Arms Race Interactions

Several lines of evidence indicate proviral segment variation among BVs has evolved in response to the arms race interactions that occur between wasps and hosts. Field surveys show BV-carrying wasps parasitize only one or two host species (48, 109, 110), and laboratory experiments indicate parasitism of a given host species by a given wasp depends on coinfection by the wasp's own BV (111, 112). BV virulence gene families show signatures of positive selection indicative of different activities and interactions with different variants of host genes (67, 113–115). Several gene family members have also diversified in terms of when and in what host tissues they are expressed (52, 113, 116–118).

Proviral genome analyses of BVs in *Glyptapanteles*, *Cotesia*, and *Microplitis* wasps all identify proviral segments that have tandemly duplicated, which is one mechanism by which segment and associated gene diversity has arisen (63, 87, 88). Other mechanisms may include recombination between homologous sequences, the activity of transposons, or the reintegration of circularized segments into the wasp's own genome through the same HIM-mediated mechanism that occurs in parasitized hosts (66, 119, 120). Selection has most likely favored segmentation of the DNAs packaged into virions because it provides a means for adjusting gene dosage (18, 21). In essence, PDVs cannot replicate in hosts, but differential amplification of segments during replication in wasps causes certain segments and their associated genes to be more abundant than others, which in turn correlates with higher transcript and protein abundance for genes on high-copy versus low-copy segments (56, 62). Functional assays further show this greater abundance is essential to the role these genes play in parasitism (56, 114, 121, 122).

More than 30 virulence gene families have been identified from different BVs (21, 23, 65). Some are shared across several subfamilies of wasps, whereas others are uniquely associated with BVs in a particular wasp genus. Gene family size also differs. This variation reflects two processes: (*a*) gene acquisition from different sources and (*b*) gene family diversification after acquisition. The former has clearly occurred at different times and from different sources in BV evolutionary history. The largest families in BV genomes are the *ank* (ankyrin repeat) and *ptp* (protein tyrosine phosphatase) genes, which are related to genes present in eukaryotes (23, 65, 113, 117, 123). All BVs except those associated with wasps in the subfamily Cheloninae have *ank* and *ptp* genes, which indicates either that they were acquired after divergence of the Cheloninae 85 million years ago or

that they were possibly present in the ancestral nudivirus and lost by chelonine BVs. In contrast, a sugar transporter family known only from BVs in *Glyptapanteles* wasps and the *egf* family of small serine protease inhibitors known only from BVs in *Microplitis* wasps are recent acquisitions that derive from translocation of wasp genes into proviral segments (63, 124). Some virulence genes have also been acquired by horizontal gene transfer from other organisms (23, 65, 125, 126). How genes translocate into BV proviral segments is unknown, although a few show signatures of acquisition by reverse transcription of an insect gene, retrovirus-mediated integration, or integration mediated by other transposable elements (60, 63, 127, 128). Diversification into multimember families has occurred through a combination of proviral segment duplication and tandem gene duplications within segments (23, 66). The presence of pseudogenes among family members also supports a "birth and death" model for virulence gene evolution, which results in gene families in different species expanding and contracting in response to adaptations by hosts (67, 113, 115, 117, 129). Recent studies indicate venom and other products wasps introduce into hosts have similarly diversified for functions that complement, rather than overlap, those of BVs (124).

Bracovirus Functions in Immunosuppression of Hosts

Insects have an innate immune system that consists of humoral and cellular elements (130–132). The primary defense against parasitoids is encapsulation, which involves binding of hemocytes to wasp eggs or larvae and thereby killing the parasitoid (133–135). Several receptors regulate hemocyte binding to foreign objects, including integrins that mediate cell-cell adhesion in capsules (132, 135–138). Hemocytes then remodel their cytoskeleton through Rho GTPase activity and placode formation by FAK (focal adhesion kinase), which results in a sheath of tightly bound cells (139). Encapsulation is also associated with activation of (*a*) the Toll and immune deficiency (IMD) pathways, which use NFκB transcription factors to regulate antimicrobial peptide expression and other functions (140–143), and (*b*) the phenoloxidase (PO) cascade, which produces reactive oxygen species and melanin implicated in killing encapsulated targets (144, 145).

BV genes disrupt multiple elements of the host's immune system. Although BV gene content varies among species, one general pattern is that BV genes tend to disrupt host regulatory pathways that control the production of effector molecules rather than the actual effector molecules that kill wasp offspring. In the case of *M. demolitor*, MdBV suppresses encapsulation and phagocytosis by directly infecting hemocytes and expressing the *glc* gene family and two members of the *ptp* family (117, 146–148). MdBV inhibits the Toll and IMD pathways through *ank* genes that are IκB mimics (149, 150) and the PO cascade through two *egf* gene family members that block PO activation (114, 121, 122). The *glc* and *egf* genes are known only from BVs in the genus *Microplitis*. As previously noted, however, *ptp* and *ank* genes are present in other BVs, and studies implicate both in disrupting hemocyte function and NFκB signaling (151, 152); *ank* genes are also present in IVs, with a recent study also showing some family members function as IκBs (153). Members of the *EP1-like* gene family, a histone H4–like gene, and a gene called *CrV1* that is present in BVs from wasps in the genus *Cotesia* have also been implicated in immunosuppression of hosts (154–156).

Bracovirus Functions in Alterations of Host Growth

PDV infection usually prolongs the larval stage, reduces host size, and inhibits metamorphosis (51). Studies with BVs from wasps in the genera *Cotesia*, *Microplitis*, and *Chelonus* show these alterations occur in part because infection alters the titers of hormones that regulate molting of host insects (157–161). Infection also causes metabolic alterations, which enhance nutrient availability to the parasitoid larva at the expense of host tissues (160, 162–164). Largely unknown are the BV genes responsible for these alterations or their molecular targets. Falabella et al. (165) report that some

Rho GTPases: a family of small G proteins that regulate intracellular actin dynamics in eukaryotic cells

Toll pathway: an arthropod homolog of the mammalian p65 NFκB signaling pathway

Immune deficiency (IMD) pathway: an arthropod homolog of the mammalian p105 NFκB signaling pathway

Antimicrobial peptides: diverse peptides capable of antagonizing or killing bacteria, select other microbes, and parasites

Phenoloxidase (PO) cascade: an immune defense pathway in arthropods consisting of serine proteases and other proteins that activate phenoloxidases

IκB family: a family of proteins containing ankyrin repeats that bind NFκBs and keep them in an inactive state in the cytoplasm

ptp family members from *Toxoneuron nigriceps* BV are expressed in the endocrine cells that produce the insect molting hormone (ecdysone). Two novel genes from *Chelonus inanitus* BV prevent hosts from pupating by an unknown mechanism (117), and select PTPs and a gene product (CpBV15β) from *Cotesia plutellae* BV inhibit host metamorphosis by different mechanisms (166, 167).

PERSPECTIVES

Progress over the past 10 years has provided many insights into PDV biology, including key features underlying the evolution of these viruses into vertically transmitted, obligate mutualists. Though not the focus of this review, several applications to biotechnology and pest management are also provided by PDVs (20, 23). More broadly, how common are mutualistic viruses? Facultative mutualisms appear to be relatively widespread, with several animals, plants, and microbes deriving fitness benefits from associations with a number of different viruses (8, 9). Most examples of conditionally beneficial mutualists also involve interactions in which a host benefits from a virus antagonizing competitors or one organism benefits from a virus affecting another.

In contrast, PDVs currently provide the only well-supported example of viruses evolving into obligate mutualists. The genomes of phage-derived gene transfer agents (GTAs) exhibit several features similar to BVs, but how GTAs benefit their bacterial hosts remains unclear (12, 22). Select poxviruses, ascoviruses, and other unclassified viruses have been described from taxa of parasitoid wasps lacking PDVs, and these viruses have potential roles in parasitism (24, 168). Too few details are known to determine whether these agents fully depend on wasps for transmission or whether they are essential for wasp survival in hosts. Nonetheless, they exhibit features that suggest they may be obligate mutualists. Several types of virus-like particles lacking nucleic acid have also been identified from parasitoids, but whether these entities are of virus origin is also unknown (169). The bias in parasitoid-associated examples suggests the life cycle of these insects may predispose them toward forming beneficial associations with viruses for the exploitation of hosts. It could also reflect that many parasitoid wasps are well studied because of their importance in pest management. A number of other parasites in diverse taxa have similar life histories as parasitoid wasps but are very poorly studied, which suggests that obligate mutualisms with viruses could be much more pervasive than currently realized.

SUMMARY POINTS

1. Polydnaviruses are large, double-stranded DNA viruses that have evolved into obligate mutualists of parasitoid wasps.
2. Polydnaviruses are vertically transmitted by wasps as integrated proviruses and function as gene delivery vectors that wasps use to genetically manipulate host insects.
3. Polydnaviruses in the genus *Bracovirus* evolved approximately 100 million years ago from a nudivirus, whereas the origin of the genus *Ichnovirus* remains unclear.
4. Bracovirus proviral genomes are dispersed in the genomes of wasps.
5. Bracoviruses share essential replication gene functions with their ancestors.
6. Polydnaviruses have acquired virulence genes with roles in parasitism from diverse sources.
7. Bracovirus DNAs in virions rapidly integrate into the genomes of parasitized host insects.

8. Bracovirus virulence genes benefit wasps by altering the immune system and growth of host insects.

FUTURE ISSUES

1. Given the known origin of bracoviruses, key needs for ichnoviruses include identifying their origin, characterizing the genes that regulate their replication, and determining whether the viruses in banchine and campoplegine ichneumonids derive from a common or different ancestor.
2. Because current data on bracoviruses derive primarily from wasps in the subfamily Microgastrinae, comparative data are still needed from other subfamilies to fully understand how bracovirus genomes are organized and function.
3. The Polydnaviridae family is not a natural taxon and needs to be reorganized.
4. What restricts bracovirus (and ichnovirus) replication to calyx cells and prevents the proviral genome from activating in other cells in females or males?
5. What are the key features that allow virulence genes to be transcribed in the host insects wasps parasitize but prevent these genes from being transcribed in wasps?
6. What are the molecular mechanisms that regulate the integration of circularized bracovirus DNA segments into the genomes of host insects?
7. How do the mutations, gene gains, and gene losses in the encapsidated form of bracovirus genomes interact with other factors to determine the host range of parasitoid wasps?
8. Are there other taxa outside of currently known subfamilies that carry polydnaviruses? What are the origins and functions of other viruses that appear to have evolved functionally similar mutualisms with parasitoid wasps?

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