Chapter 9

Unusual Viral Genomes: Mimivirus and the Polydnaviruses

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SUMMARY

Recent publication of some large dsDNA viral genome sequences has challenged the ways in which we view viral classification and evolution. Here, I focus on comparative analysis of the genomes of two of these viruses. The first is the amoebal virus Mimivirus, which has the largest viral genome sequenced to date and shows complexity comparable to some small parasitic prokaryotes. The second virus is the polydnavirus, which shares a unique symbiotic relationship with parasitoid wasps, in which the virus is entirely dependent on the wasp for replication and in return viral gene expression promotes parasitoid survival. While the genomes of these two viruses share few homologous features, in parallel ways they have challenged our definition of viruses and illuminated new avenues in which viruses interact with their hosts and the environment over an evolutionary timescale.

INTRODUCTION

Over the past decade, a number of viral genome sequences have been published which have altered the way in which we view the definition and evolution of viruses. Large dsDNA viral genomes have shown complexity comparable to some small parasitic prokaryotes, and the biologies of these viruses have illuminated new ways in which viruses interact with their hosts and the environment over an evolutionary timescale. In 2005, I coauthored a review (Desjardins *et al.*, 2005) in which we briefly discussed the genomes of two viruses that are drastically changing the way we look at viruses.

One of these viruses was the polydnavirus, which shares a symbiotic relationship with parasitoid wasps. Virions are manufactured solely in the female wasp's reproductive tract from proviral DNA, and virions themselves do not encode the capacity for replication or packaging. When polydnavirus-containing wasps inject eggs into their caterpillar host, they co-inject virions which, through suppression of the caterpillar immune system, act to ensure the survival of the young parasitoid. These viruses persist only by being transmitted vertically in their primary wasp host as proviral DNA, calling into question even the classification of polydnaviruses as viruses. Polydnavirus genomes are in many ways more eukaryote-like than any other virus, as the heritable form of the virus lies within a eukaryotic genome.

The other virus we examined was the amoebal virus Mimivirus, and at 1.2 Mbp, the Mimivirus genome is the largest viral genome sequenced to date (Raoult *et al.*, 2004). The Mimivirus genome rivals in both size and complexity the genomes of many small parasitic prokaryotes. This has even led scientists to propose the term 'girus' for giant viruses which show complexity comparable to or greater than these prokaryotes (Claverie *et al.*, 2006). While the Mimivirus genome lacks some of the eukaryote-like features of polydnavirus genomes, it does encode a level of translational machinery never before seen in viruses. Furthermore, Mimivirus relatives are the only viruses known to have their own viruses (virophages), opening up new avenues for virus–virus interactions. The polydnavirus and Mimivirus genomes, in different ways, have pushed the envelope of both viral complexity and the relationships of viruses with their host and host environment.

In part, the purpose of this chapter is to compare the genomes and biology of Mimivirus and the polydnaviruses. However, the other purpose of this chapter is to bring the discussions centered around Mimivirus to the polydnavirus researcher. Unfortunately, it seems that polydnaviruses are often left out of discussions on 'giant' viruses, despite the fact that many polydnavirus genomes fall well above the most stringent 280–300-Mbp minimum size required

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for classification as giant viruses (Claverie *et al.*, 2006; Van Etten *et al.*, 2010). In many articles on giant viruses, the existence of polydnaviruses is mentioned initially, and then it is stated that they will 'not [be] discussed further' (e.g., Claverie *et al.*, 2006). This appears to be due to the polydnavirus genome's eukaryote-like low coding density, which is argued to make comparisons to other viruses difficult. Regardless, many of the questions and debates Mimivirus has brought to the field of viral evolution are also quite relevant to polydnavirus research. First, I will introduce the genomes of Mimivirus and its virophage Sputnick, followed by the polydnavirus genomes. I will then compare these genomes and discuss the implications of these genome sequences for our understanding of the definition and evolution of viruses.

MIMIVIRUS: THE LARGEST SEQUENCED VIRUS

Mimivirus is a virus of the amoeba *Acanthamoeba polyphaga* and was first isolated from a cooling tower in England in 1992 (La Scola *et al.*, 2003). Gram-positive staining and the large size of Mimivirus led its discoverers to believe it was a bacteria for over ten years. In 2003, it was renamed Mimivirus, meaning 'mimicking microbe'. The virus particle is surrounded by an isocahedral capsid with a diameter of 500 nM, which is then covered by a thick layer of fibrils giving Mimivirus a 'hairy' appearance and an overall diameter of 750 nM (Xiao *et al.*, 2005). Due to their enormous size, Mimivirus particles do not fit though the 0.2 μM filter typically used to isolate viruses.

The replication cycle of Mimivirus is shown in Fig. 1. It begins when a virion is phagocytized by an amoeba. The binding of lysosomes to the Mimivirus-containing phagosome triggers the opening of the capsid, probably through lysosomal activity, which then fuses to the phagosome membrane and releases viral DNA in the cytoplasm (Zauberman *et al.*, 2008). It is then hypothesized that Mimivirus DNA enters the amoebal nucleus, as fluorescent staining of this time-point shows an increase in nuclear AT content, which could correspond to the AT-rich Mimivirus DNA (Suzan-Monti *et al.*, 2007). This may be indicative of a transient nuclear DNA replication phase as seen in asfarviruses. Subsequent to the entry of Mimivirus DNA into the nucleus, large virus factories develop in the cytoplasm which subsequently package new Mimivirus particles. These viral factories appear centered around a compartment the size of a Mimivirus virion, which could indicate replication and packaging machinery similar to poxviruses, where transcription of early genes occurs immediately following infection from within the virion (Claverie and Abergel, 2009). Mimivirus particles are then released through cell lysis.

The Mimivirus genome is the largest viral genome sequenced to date, composed of a single linear dsDNA molecule of ~1.18 Mbp and predicted to encode 911 protein-coding genes (Raoult *et al.*, 2004). Additional transcript sequencing increased the number of predicted genes to 960 and identified 26 non coding RNAs (Legendre *et al.*, 2010). The Mimivirus genome encodes a number of genes never before seen in viruses, such as an unprecedented number of genes involved in protein translation: four aminoacyl-tRNA synthetases, five translation factors, and the first identified viral homolog of a tRNA modification enzyme (Raoult *et al.*, 2004). Furthermore, Mimivirus encodes five DNA repair genes, three topoisomerases, three protein chaperones, and at least eight genes involved in the synthesis and modification of proteins and polysaccharides. None of these functional classes of genes has been identified in this number in any previously sequenced viral genome. However, like all viruses, the Mimivirus genome does not encode any ribosomal proteins and cannot replicate without host replication machinery.

Mimivirus genes show an unprecedented level of promoter conservation—45% of all Mimivirus genes have a perfectly conserved AAAATTGA motif within 150 bp of their start site (Suhre *et al.*, 2005). It is hypothesized that this motif is functionally equivalent to the eukaryotic TATA box. This appears to be a unique feature of Mimivirus, as related viruses do not show a similar level of conservation of this or any other promoter element.

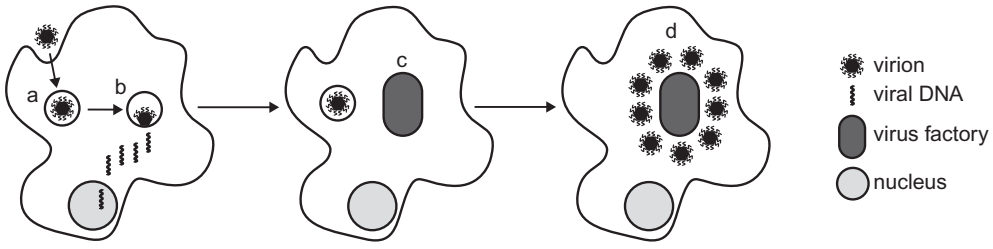

FIGURE 1 Replication cycle of Mimivirus. A: Mimivirus is phagocytized by the amoeba and sequestered in a phagosome. **B:** The Mimivirus virion releases DNA into amoebal cytoplasm, and may enter the nucleus. **C:** Virus factories appear in the cytoplasm of the infected amoeba. **D:** Mature Mimivirus virions are produced by the virus factory, and subsequently released by lysis of the amoebal cell.

Furthermore, most Mimivirus mRNAs have a 3′ polyadenylation signal which forms a perfectly conserved hairpin structure (Byrne *et al.*, 2009). This degree of transcriptional complexity has not been found in any other virus.

Mimivirus is classified as a nucleo-cytoplasmic large DNA virus (NCLDV) (La Scola *et al.*, 2003) and encodes all nine of the class I core genes assigned to NCLDVs (Iyer *et al.*, 2001; Raoult *et al.*, 2004). NCLDVs were named as such because they either replicate entirely in the cytoplasm (e.g., asfarviruses, iridoviruses, poxviruses) or begin replication in the nucleus and end replication in the cytoplasm (e.g., phycodnaviruses) (Iyer *et al.*, 2001). Also, as the name implies, NCLDVs have large genomes, generally over 100 kbp, although inclusion in the group is phylogenetic rather than phenotypic. NCLDVs attack a wide range of eukaryotic hosts ranging from algae (phycodnaviruses) to insects and vertebrates (e.g., poxviruses). Based on phylogenetic analysis of the NCLDV core genes, Mimivirus was placed in its own family Mimiviridae (La Scola *et al.*, 2003), and further phylogenetic analysis has supported this classification (Iyer *et al.*, 2006; Raoult *et al.*, 2004).

Numerous Mimivirus relatives have been identified through environmental sequencing of ocean samples (Ghedin and Claverie, 2005; Monier *et al.*, 2008) and targeted culture from fresh water environmental samples (La Scola *et al.*, 2010). Mamavirus, which was isolated from amoebas in France and has a slightly larger capsid than Mimivirus, has a similar genome size of ~1.2 Mbp (La Scola *et al.*, 2008). It is closely related to Mimivirus, as 99% of Mamavirus open reading frames (ORFs) have 75–100% sequence identity with orthologs in Mimivirus. Mimivirus' ocean-dwelling relatives certainly attack a range of hosts beyond amoebas, and Claverie *et al.*, (2009) hypothesize that this host range includes such disparate organisms as algae, corals, and sponges. These relatives may have even larger genomes than Mimivirus, as viruses with larger capsid sizes to Mimivirus have been isolated, and capsid size is often reflective of genome size (La Scola *et al.*, 2010). However, it remains to be seen how much overlap exists between the coding capacity of the Mimivirus genome and the genomes of these currently uncharacterized viruses, and whether or not these new viruses will stretch our definition of viruses.

SPUTNIK: A VIRUS OF A VIRUS

Mimivirus and its relatives are even complex enough to have their own viruses. Sputnik, which infects the Mimivirus relative Mamavirus, in addition to the Mimivirus itself, has been termed a 'virophage' (La Scola *et al.*, 2008). Sputnik is much smaller than Mimivirus, only 50 nM in diameter, and has a small circular dsDNA genome of 18.3 kbp, encoding only 21 proteins, and is 73% AT. Only eight of the proteins have detectable homologs in viruses, bacteria, or eukaryotes; three of these genes have identifiable homologs in Mimivirus. Given the chimeric nature of this small genome, it is currently unknown what other viruses Sputnik may be related to.

Sputnik can only replicate in virus factories produced during an amoeba's infection with Mimivirus, and replicating is deleterious to the replication of Mimivirus by causing defective capsid formation, resulting in a significant decrease in the rate of amoebal lysis. In some ways, Sputnik is similar to satellite viruses, which utilize the factories of co-infected viruses to assist with their own packaging (Desnues and Raoult, 2010). However, while some satellite viruses may reduce the replication rate of their host virus, Sputnik is the first virus described to cause it's host virus to create defective particles. Also, satellite viruses generally replicate their DNA in the host cell nucleus whereas Sputnik replicates within the virus factory. Furthermore, the presence of Mimivirus-like hairpin structures in 14 intergenic regions in the Sputnik genome suggest that Sputnik is using Mimivirus machinery for replication and is therefore more than a satellite virus (Claverie and Abergel, 2009). Together, these observations provide convincing evidence that Sputnik is more than a satellite and is a virus of a virus.

POLYDNAVIRUSES: VIRAL SYMBIONTS

Mimivirus and Sputnik showed us new levels of viral genome complexity and new types of virus–virus interactions, but the sequencing of the first polydnavirus genome opened up a different set of questions regarding the limits of viral complexity and virus–eukaryote interactions. The polydnavirus replication cycle is shown in Fig. 2. The polydnavirus genome is integrated into the parasitoid wasp genome. Replication of proviral segments occurs only in the nuclei of calyx cells in the reproductive tract of the female wasp, followed by excision and circularization of viral genome segments from the amplified proviral DNA. Virions are subsequently produced utilizing a virion packaging system present in flanking wasp DNA (Bézier *et al.*, 2009a). During packaging, nuclear pores increase in abundance and the cytoplasm of the calyx cells fill with ribosomes, suggesting that viral structural proteins are synthesized in the cytoplasm and then imported into the nucleus (Wyler and Lanzrein, 2003). In bracoviruses, virions are released by lysis of the calyx cells into the oviduct lumen (Wyler and Lanzrein, 2003), while in ichnoviruses virions are released by budding (Volkoff *et al.*, 1995). The female wasps then co-inject polydnavirus virions along with eggs into their caterpillar hosts. The viruses then enter caterpillar host cells where they act to suppress the host immune response and alter host physiology, promoting survival of the young parasitoid.

The first genome sequence of a packaged polydnavirus was that of *Cotesia congregata* bracovirus (CcBV) (Espagne *et al.*, 2004). This genome is composed of 30

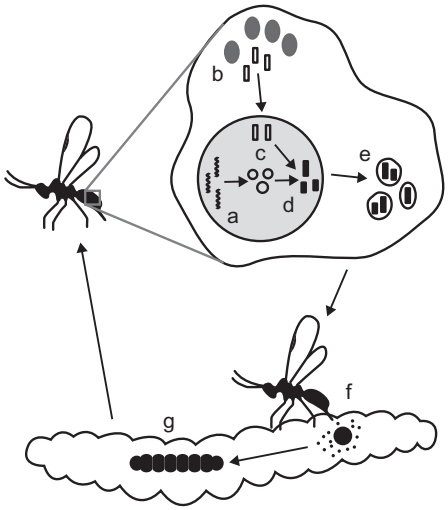

FIGURE 2 Replication cycle of a polydnavirus (adapted from (Desjardins *et al.*, 2005)). A: Replication of proviral DNA occurs only in the nuclei of calyx cells in the reproductive tract of the female wasp. **B:** Simultaneously, viral structural proteins are generated in the cytoplasm. **C:** Viral DNA is excised and circularized, and viral structural proteins are imported into the nucleus. **D:** Virus DNA is encapsidated. **E:** Virions are generated by budding from the nucleus and released by lysis (in bracoviruses) or a second budding (in ichnoviruses). **F:** Female wasps co-inject virions along with eggs into their caterpillar hosts. Virions enter caterpillar host cells where they act to suppress the host immune response and alter host physiology, promoting survival of the growing young parasitoid and therefore survival of proviral DNA (G).

circular dsDNA segments totaling ~560 kbp and is predicted to encode 156 protein-coding genes. Polydnaviruses are unique among viruses in having their genome broken into multiple segments (hence the name 'poly-DNA-virus'). A number of packaged polydnavirus genome sequences from both the bracovirus and ichnovirus lineages followed: MdBV and CsIV (Webb *et al.*, 2006), HfIV and TrIV (Tanaka *et al.*, 2007), GfIV (Lapointe *et al.*, 2007), and GiBV and GfBV (Desjardins *et al.*, 2008). These genomes range in segment number from 15 in MdBV to 105 in GfIV and in size from 189 kbp in MdBV to 589 kbp in GfBV. Ichnoviruses typically encode a larger number of smaller circular segments than bracoviruses. The polydnavirus proviral genome is integrated into the parasitoid wasp genome at multiple loci, each containing one to many proviral genome segments (Annaheim and Lanzrein, 2007; Desjardins *et al.*, 2008).

Polydnavirus genomes are unusual among viruses in that they encode large gene families, and the CcBV genome encodes a number of these previously identified as virulence factor in non viral pathogens, including protein tyrosine phosphatases, inhibitors of NF-κB (also called vankyrins (Kroemer and Webb, 2005)), cysteine-rich proteins, EP1 (early protein 1) genes, and cysteine protease inhibitors (cystatins) (Espagne *et al.*, 2004). Webb *et al.* (2006) identified a number of gene families unique to ichnoviruses, including rep genes, viral innexins (vinnexins), plus two unique gene families in MdBV: egf-motif (epidermal growth factor) genes and glc genes (mucins). Further polydnavirus genome sequencing identified new and sometimes taxon-specific genes families with functional predictions, e.g., sugar transporters in GiBV and GfBV (Desjardins *et al.*, 2008). In fact, polydnavirus gene families are remarkably taxon-specific, and the only gene family that appears to be shared across bracoviruses and ichnoviruses is the vankyrins (Falabella *et al.*, 2007; Webb *et al.*, 2006).

Conservation of gene families within polydnavirus lineages is more variable. Campoplegine ichnovirus genomes appear to have a large overlap between gene family content (Tanaka *et al.*, 2007), while bracoviruses do not (Webb *et al.*, 2006). The gene family content of the banchine ichnovirus GfIV includes the ubiquitous vankyrins, the bracovirus protein tyrosine phosphatases, and a previously undescribed NTPase-like gene family with similarity to NCLDV NTPases (Lapointe *et al.*, 2007). However, only having a single banchine ichnovirus genome sequence available makes drawing generalities about the group's gene content difficult. Some features appear to be more conserved within polydnavirus lineages than packaged genes, including the packaging machinery (Bézier *et al.*, 2009a) and motifs governing segment excision (Desjardins *et al.*, 2007).

Polydnavirus genomes have a remarkably low coding density, ranging from 20–30% (see Table 1). In fact, some ichnoviruses have entire genome segments which are not predicted to encode any proteins; the function of these segments is currently unknown (Tanaka *et al.*, 2007). This situation is most extreme in the banchine ichnovirus GfIV, where 30% of the genome segments contain no identifiable gene (Lapointe *et al.*, 2007). Despite this eukaryotic-like low coding density, polydnavirus genomes do have some properties which may be indicative of an external viral origin, including atypical nucleotide composition and simpler gene structures compared to flanking wasp DNA (Desjardins *et al.*, 2008, 2007).

Perhaps the most unusual feature of polydnavirus genomes, that which calls their classification as viruses into question, is their complete lack of replication and packaging genes. The exception to this is the CsIV structural protein p12, which is encoded in the packaged ichnovirus genome (Deng and Webb, 1999), although a single structural protein is certainly not enough to replicate and package an entire virus. Recently, Bézier *et al.* (2009a) discovered nudivirus-derived packaging machinery within the genomes of the polydnavirus-carrying wasps *Cotesia congregata* and *Chelonus inanitus*. This included genes involved in transcription, packaging and assembly, and envelope components. In total, genes representing 22

TABLE 1 Morphological and Genomic Features of Mimivirus, its Virophage 'Sputnik', and Polydnaviruses from the Three Major Lineages: Bracoviruses (*Cotesia Congregata* Bracovirus, CcBV), Camplopegine Ichnoviruses (*Hyposoter Fugitivus* Ichnovirus, HfIV), and Banchine Ichnoviruses (*Glypta Fumiferanae* Ichnovirus, GfIV)

Feature	Mimivirus	Sputnik	CcBV	HfIV	GfIV
Capsid size (nM)	500	50	35 × 30–150	85 × 330	30 × 125
Capsid shape	Isocahedral	Isocahedral	Rod	Lenticular	Rounded rod[1]
Capsids per virion	One	One	Multiple	One	Multiple
Envelopes	One	None	One	Two	Two
Replication site	Cytoplasm	Virus factory	Nucleus	Nucleus	Nucleus
Genome size (kbp)	1181	18	568	246	291
Genome segments	1	1	30	56[2]	105
Genome orientation	Linear	Circular	Circular	Circular	Circular
% AT	72	73	66	57	63
% coding density	91	80	27	30	20
Packaged genes	960	21	155	143	103
Unpackaged genes	0	0	19	?[3]	?
tRNA genes	6	0	7	0	0
Non coding RNAs	26	0	0	0	0

Unless otherwise stated, features refer to the packaged viral genome.
[1]*GfIV capsids appear intermediate in shape between bracovirus and campoplegine ichnovirus virions, forming a rounded rod (Lapointe* et al.*, 2007).*
[2]*Eleven of the 56 genome segments in HfIV can generate smaller nested segments, further adding to genome complexity (Tanaka* et al.*, 2007).*
[3]*Twenty unpackaged genes were identified in the related* Hyposoter didymator *ichnovirus (Volkoff* et al.*, 2010).*

nudivirus genes were identified; 19 in *Cotesia congregata* and 18 in the distantly related *Chelonus inanitus*. Through mass spectrometry and Q-PCR experiments, Bézier *et al.* provided substantial evidence that the nudivirus-related genes in the wasp genome are the packaging machinery for their associated bracoviruses. While Bézier *et al.* 2009a identified numerous genes involved in virion packaging and transcription, they did not find any viral DNA replication genes, suggesting that host genes perform this function. Viral packaging machinery has also been recently identified for *Hyposoter didymator* ichnovirus (Volkoff *et al.*, 2010). As with the bracovirus packing machinery, the genes involved in viral particle production were clustered in the wasp genome. Unlike the bracovirus machinery, however, the genes involved shared no similarity with any known viruses.

GENOME FEATURES SHARED ACROSS MIMIVIRUS AND POLYDNAVIRUSES

A comparison of the genomes of Mimivirus, Sputnik, and representatives of the three lineages of polydnaviruses can be seen in Table 1. At first glance, it would appear that little is shared between Mimivirus and the polydnaviruses other than both being encoded by large, AT-rich dsDNA genomes. Comparative genome analysis of NCLDVs found that polydnaviruses and NCLDVs 'hardly share any homologous proteins' (Iyer *et al.*, 2006). One rare example is a DNA Polymerase B2 domain in CcBV, although it appears that this domain is a component of a Maverick transposable element rather than of viral origin (Drezen *et al.*, 2006). However, one might not expect to find genes in common given that the most conserved viral genes belong to functional categories absent from the packaged polydnavirus genomes published at the time, such as those involved in viral replication and packaging.

Utilizing recently published transcriptional data for Mimivirus (Legendre *et al.*, 2010), and polydnavirus packaging machinery identified for both CcBV and HdIV (Bézier *et al.*, 2009a; Volkoff *et al.*, 2010), I queried the Mimivirus proteome with the polydnavirus packaging genes using BLASTP with a cutoff of 1e-5. No hits were identified, reinforcing the idea that neither bracoviruses nor ichnoviruses are closely related to Mimivirus. Querying environmental metagenomic data with Mimivirus genes has revealed a diversity of Mimivirus-like sequences (Ghedin and Claverie, 2005), so I similarly queried Genbank's environmental protein sequence database with the polydnavirus

packaging genes as above. No significant matches were found, although the marine environment, from which a large quantity of environmental sequence data originates, is less ideal for searches of polydnavirus relatives than it is the relatives of protist and algae viruses.

One genome feature shared by Mimivirus and some polydnaviruses is the encoding of tRNA genes, although the exact function of these genes has not been proven in either virus. All four sequenced bracovirus genomes are predicted to encode from three to seven tRNAs each, while only one ichnovirus genome, that of TrIV, was predicted to encode a single tRNA. In Mimivirus, tRNAs appear to be transcribed in a unique way. In eukaryotes, tRNA transcripts are generated in the nucleus using RNA polymerase III. However, Mimivirus both lacks RNA polymerase III and also replicates entirely in the cytoplasm. This led to Byrne *et al.*'s (2009) discovery that Mimivirus tRNA transcripts are polyadenylated, sometimes in pairs and in one case paired with a protein-coding gene, suggesting that Mimivirus tRNAs are likely transcribed by the same machinery as protein-coding genes. The most obvious purpose of these viral tRNAs would be to adjust the difference in codon and/or amino acid usage from that of the virus to that of the host, as has been hypothesized for phages (Bailly-Bechet *et al.*, 2007), but thus far no convincing evidence has been put forth to support this idea for either Mimivirus or polydnaviruses.

A second feature shared by Mimivirus and polydnaviruses which is unusual for viruses in general is the presence of introns. Mimivirus contains a small number of self-splicing introns and inteins, which are self-splicing regions of a protein. The related phycodnaviruses also encode both self-splicing introns and inteins (Wilson *et al.*, 2009), suggesting that both of these viruses may have acquired them prior to a common ancestor. While transcriptional data have verified the existence of introns in at least some polydnavirus genes (Desjardins *et al.*, 2007; Webb *et al.*, 2006), the exact degree of introns present in polydnaviruses is still up for debate. Espagne *et al.* (2004) predicted that 69% of CcBV genes contained introns, while Webb *et al.* (2006) reannotated the same data and predicted 7% contained introns. Given the limited transcriptional data available for polydnavirus genes, this difference is largely the result of whether the gene predictor used incorporates introns or not. However, even when widespread introns are predicted in polydnavirus genes, such as for GiBV and GfBV, polydnavirus genes appear to have fewer introns on average than flanking wasp genes, implying in some sense that even intron-containing polydnavirus genes are simpler than their wasp counterparts (Desjardins *et al.*, 2008).

Perhaps one of the most intriguing elements of both the Mimivirus and polydnavirus genomes is what they don't tell us—in total these genomes encode an enormous number of proteins of completely unknown function, with no similarities to any proteins in unrelated viruses. Here, I used BLAST2GO (Conesa and Gotz, 2008) with a relaxed 1e-5 cutoff to functionally annotate the proteomes of Mimivirus and CcBV with Gene Ontology (Ashburner *et al.*, 2000) terms. Functional annotations were assigned to only 42% of Mimivirus genes and 24% of CcBV genes. Extensive laboratory studies will need to be conducted in order to begin to understand the functions of this wide array of currently unannotated viral genes.

MIMIVIRUS ORIGINS

There has been much discussion and debate over the origins of unusual large dsDNA viruses such as Mimivirus and the polydnaviruses. A good starting point for discussion is a phylogeny of large dsDNA viruses estimated by Iyer *et al.* (2006) by comparative analysis of a large number of viral genomes (Fig. 3). They divide NCLDVs into two major lineages: one containing poxviruses and asfarviruses, and one containing mimiviruses, phycodnaviruses, and iridoviruses. They argue that many of the unique features of Mimivirus are derived and that it likely evolved from a smaller ancestral NCLDV. They further hypothesize that this ancestral NCLDV and other large DNA viruses evolved from prokaryotic viruses shortly after the emergence of the eukaryotic cell, based on phylogenetic analyses suggesting eukaryotic origins of a large number of core viral genes.

Iyer *et al.*, 2006 cannot determine if all large dsDNA viruses share a common ancestry, or evolved independently and converged when faced with similar selective pressures, although they favor the former hypothesis. They hypothesize that NCLDVs and baculoviruses have nonhomologous virion packaging systems, which suggests that any baculovirus-related polydnavirus packaging machinery cannot be phylogenetically compared to that of Mimivirus. Furthermore, they hypothesize that while that NCLDVs and baculoviruses share a homologous DNA replication system, the systems are not orthologous, i.e., they did not originate from a common ancestor, and therefore the replication systems of Mimivirus and polydnaviruses may also not be comparable. Alternate hypotheses for phylogenetic relationships between large dsDNA viruses have been proposed, such as all viruses with isocahedral capsids forming a monophyletic group (see Krupovic and Bamford (2008) for a review of this hypothesis). However, given the different capsid shapes of Mimivirus, bracoviruses, and ichnoviruses, this hypothesis also fails to provide phylogenetic relationships between these viruses.

An ancestral NCLDV is not the only origin hypothesized for Mimivirus. Using phylogenetic analysis, the authors of the Mimivirus genome sequence hypothesize that Mimivirus represents a branch of life basal to the eukaryotes, the product of reductive evolution from a more complex ancestor, and should be considered the fourth domain of life (Raoult

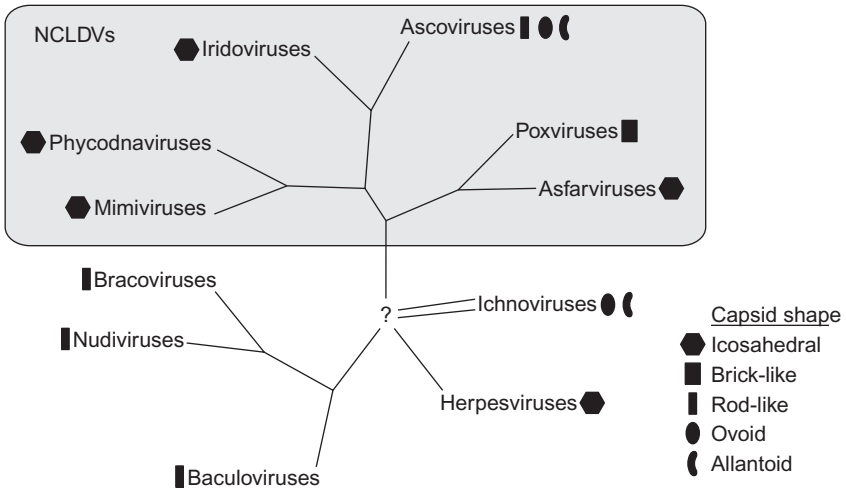

FIGURE 3 Phylogeny of large dsDNA viruses showing the relative placement of Mimivirus and polydnaviruses (adapted from Iyer *et al.*, 2006 to include the supported placement of bracoviruses (Bézier *et al.*, 2009a)). While it was originally hypothesized that ichnoviruses are related to ascoviruses (Bigot *et al.*, 2008; Federici and Bigot, 2003), ichnovirus structural proteins show no similarity to structural proteins of ascoviruses or any other viruses, leaving the origins of ichnoviruses unknown (Volkoff *et al.*, 2010). Two lines are shown leading to the ichnovirus lineage, representing the two hypothesized origins of ichnoviruses (Lapointe *et al.*, 2007). Diversity of capsid shapes within each group is also depicted.

et al., 2004). Further arguing this hypothesis, Claverie (2006) stated that the presence of incomplete translational machinery implies the ancestor of Mimivirus had a complete machinery and lost components. Conserved transcriptional elements found in Mimivirus but no other virus, such as the promoter element (Suhre *et al.*, 2005), have also been used to support this hypothesis. It is not explicitly clear in this hypothesis how the other NCLDVs are phylogenetically related to Mimivirus, although presumably if Mimivirus represents the most ancestral NCLDV, the remaining NCLDVs represent derived lineages with further reduced genomes. Yet a third hypothesis for the origin of Mimivirus is that it is largely chimeric and its evolution has been driven by the acquisition of genes through horizontal transfer (Moreira and Brochier-Armanet, 2008).

These arguments over the origin of Mimivirus reflect arguments on the origins of viruses as a whole. The two primary hypotheses for viral origins are the reduction hypothesis and the escape hypothesis. In the reduction hypothesis, viruses began as complex organisms capable of self-replication, and over time evolved a parasitic lifestyle which caused them to lose the ability to self-replicate. This is similar to the hypothesis of the Mimivirus origin by Raoult *et al.* (2004). Alternatively, the escape hypothesis postulates that viruses originated from a set of viral components escaping from a more complex organism. One main difference in the predictions of these hypotheses is that in the reduction hypothesis, many viral genes have a specifically viral origin, wherein the escape hypothesis viral genes have a cellular origin. While many authors claim the lack of similarity between a large fraction of viral genes and cellular genes implies that the two do not share common ancestry (e.g., Claverie (2006)), the vast evolutionary timescale over which this has occurred makes this conclusion difficult to draw. A third hypothesis is that viruses originated during the emergence of cells from DNA replicons ancestral to prokaryotes and potentially played a role in the origin of cellular life (Forterre, 2006; Iyer *et al.*, 2006).

ORIGINS OF PARASITOID–VIRUS SYMBIOSES

The current phylogenetic placement of polydnaviruses relative to other large dsDNA viruses is shown in Fig. 3. The wasp–polydnavirus association is believed to have evolved multiple times independently, as the wasp hosts of bracoviruses and ichnoviruses lack a common ancestor (Stoltz and Whitfield, 1992). A variety of morphological differences also exist between the capsids and virions of bracoviruses and ichnoviruses (see Table 1). Relationships between polydnaviruses and other viruses were proposed based on the structure of their virions. Bracoviruses were proposed to be related to baculoviruses while ichnoviruses were proposed to be related to ascoviruses (Federici and Bigot, 2003; Whitfield and Asgari, 2003). Bézier *et al.* (2009a) uncovered the packaging machinery of bracoviruses and identified their relatives as nudiviruses, which are closely related to their previously proposed relatives, the baculoviruses. Identification of ichnovirus packaging machinery did not provide the same resolution: none of the identified packaging genes were similar to ascovirus genes or genes of any known virus (Volkoff *et al.*, 2010).

It has been further proposed that the campoplegine and banchine ichnovirus lineages arose independently (Lapointe *et al.*, 2007). This is based on the lack of a common ancestor between the polydnavirus-containing clades of campoplegine and banchine wasps, differences in virion size and structure between campoplegine and banchine ichnoviruses (see Table 1), and a gene family representation in the banchine ichnovirus HfIV that shares more with bracoviruses than ichnoviruses. If correct, this hypothesis implies three independent origins of a remarkably convergent polydnavirus system.

The lack of core viral machinery in the packaged genome sequences of polydnaviruses led to multiple hypotheses on their origins. One hypothesis was that polydnaviruses originated from a large virus which was integrated into the wasp's genome, which then underwent reductive genome evolution, having its replication and packaging machinery transferred to the wasp genome (Whitfield and Asgari, 2003). The second hypothesis was that polydnaviruses derived from wasp DNA which had captured viral structural and virulence proteins and over time accumulated additional virulence components from the wasp genome (Federici and Bigot, 2003). An alternate version of this second hypothesis specifically involves circular DNA mobile elements capturing viral structural proteins (Espagne *et al.*, 2004). This is not unprecedented as a group of ssDNA viruses known as geminiviruses are hypothesized to have evolved from a bacterial plasmid which captured a capsid-coding gene (Koonin and Ilyina, 1992; Krupovic *et al.*, 2009). A third hypothesis was that polydnavirus structural components were evolved by the wasp *de novo* rather than acquired from a virus (Federici and Bigot, 2003). However, given the extensive nudivirus-related packaging machinery discovered by Bézier *et al.* (2009a) the initial hypothesis of viral genome capture and reduction seems the most likely.

ARE MIMIVIRUS AND POLYDNAVIRUSES REDEFINING VIRUSES?

The Mimivirus genome exceeds in both sheer genome size and number of encoded proteins a large number of parasitic prokaryotes (a good comparison can be found in Ward and Fraser, (2005)). This unprecedented complexity has led much debate on the definition of viruses and their relationship to cellular life (e.g., see Moreira and Lopez-Garcia (2009) and associated correspondence for a debate over whether or not viruses should be included in the tree of life). Several new definitions of the term virus have been proposed (reviewed in Forterre (2010)). One such proposition is that viruses could be defined by encoding capsids while bacteria, archaea, and eukaryotes be defined by encoding ribosomes (Raoult and Forterre, 2008). While at first glance this definition may seem sensible, attempts to classify polydnaviruses and their wasp hosts using this definition produce strange results. Under this definition, whether or not polydnaviruses would be considered viruses depends on whether non-packaged components are considered part of the polydnavirus or wasp genome. If the polydnavirus packaging machinery is considered part of the wasp genome, then polydnavirus-associated wasps could be considered both alive (by nature of encoding ribosomes) and viruses (by nature of encoding capsids). Many parasitoid wasps also encode the ability to produce non-polydnavirus encapsidated particles, termed virus-like particles, furthering the idea that the mere ability to encode a capsid is not specific to viruses. While it may be possible to define bacteria, archaea, and eukaryotes by the presence of ribosomes, it seems that viruses cannot be defined by the presence of capsids alone. It is possible that both encoding a capsid and being packaged entirely by that capsid are required to be considered a virus—this definition would exclude parasitoid wasps from viruses.

Other definitions of viruses which have been recently proposed include the idea of viruses as viral factories (Claverie, 2006) and viruses as cellular organisms: the virocell (Forterre, 2010). Claverie (2006) argues that viewing a virus solely as a virion ignores much of the complexity of large dsDNA viruses. The viral factory is a complex intracellular structure where viral genes are transcribed and translated into proteins, and viral DNA is replicated and packaged, and that this structure shows remarkable similarity to an intracellular parasitic bacterium. However, Forterre (2010) points out that this concept only encompasses viruses which replicate in the cytoplasm, excluding a large fraction of viruses which replicate in the nucleus (including polydnaviruses). Using an extension of Claverie's logic, in which the entire cell is taken over by the virus, Forterre argues that the infected cell, or 'virocell' is the definitive form of the virus. At this point, however, the debate veers away from hypotheses based on the Mimivirus and into speculation on what should be considered life; therefore, I will not further explore this argument.

The definition debate within the polydnavirus community seems not to center around how to redefine viruses to properly account for polydnaviruses, but rather to decide if polydnaviruses should be considered viruses at all. According to the International Committee on Taxonomy of Viruses (www.ictvdb.org, accessed 11/13/2010), polydnaviruses are currently defined as viruses. However, the absence of polydnaviruses from virtually all discussion on the evolution and classification of large dsDNA viruses (e.g., Claverie *et al.* (2006), Van Etten *et al.* (2010)) implies that many viral researchers may not consider them true viruses. Already, it has been proposed that polydnaviruses should not be considered viruses but rather immunosuppressive organelles (Federici and Bigot, 2003). Certainly, being derived from a nudivirus means that polydnaviruses are viruses in a phyogenetic sense,

whereas if the structural components were not of viral origin, then polydnaviruses would be excluded from viruses. Given this, whether or not a polydnavirus is considered a virus or an organelle-like component of the wasp genome is dependent on whether or not viral packaging machinery must be included with the packaged viral DNA in order for a virion to be considered a virus.

THE OVIPOSITOR AND AMOEBA AS EVOLUTIONARY ENVIRONMENTS

Stoltz and Whitfield (2009) point out that regardless of whether or not polydnaviruses expand our definition of viruses, they should expand our view of viral evolution, in that viruses are not always antagonistic and can evolve symbiotic mutualistic relationships with eukaryotes. Symbiotic relationships between parasitoid wasps and 'free-living' viruses have already been identified, such as the relationship between the DpAV4a ascovirus and its ichneumonid wasp host, *Diadromus puchellus* (Bigot *et al.*, 2009). All polydnavirus-associated wasps are koinobiont endoparasitoids, where the host continues to develop subsequent to the injection of the parasitoid egg—this provides an ideal environment for parasitoid–virus coevolution. In this situation, the wasp, under attack from the host immune system, can benefit from viral immune suppression. The parasitoid ovipositor, in a sense nature's 'dirty needle', provides an ideal mechanism for the spread of viruses between multiple hosts.

While the parasitoid–host environment may provide an ideal situation for the development of symbiotic relations between parasitoid and virus, the amoeba provides an ideal environment for the evolution of interactions between different viruses and parasitic prokaryotes. Amoeba are known to be infected by taxonomically diverse organisms (Greub and Raoult, 2004), and the ability to co-culture viruses and prokaryotic parasites together in a single amoeba provides a potential environment for extensive gene mixture (Boyer *et al.*, 2009). The chimeric nature of the Mimivirus, Mamavirus, and Sputnik genomes have led some researchers to call the amoeba intracellular environment a 'melting pot' of gene transfer where viruses replicate in a soup of foreign DNA (Boyer *et al.*, 2009). The discovery of virophages adds another layer of complexity to these interactions, where they serve as vehicles for horizontal gene transfer between viruses. The presence of Mimivirus genes in the Sputnik genome suggests that horizontal gene transfer between the viruses has already occurred (La Scola *et al.*, 2008).

These kinds of genome–genome interactions may not be unique to a parasitoid–virus symbiosis or amoeba. A replication cycle similar to polydnaviruses has been proposed for the phycodnavirus EsV-1 (Delaroque and Boland, 2008). EsV-1 is a virus of the brown algae *Ectocarpus siliculosus*, infecting algal gametes and spores. The virus is integrated into the algal genome in a proviral state, and spreads through the developing host as a provirus through mitosis. Expression of proviral genes occurs only when triggered by environmental cues. It should be noted that while the EsV-1 genome has a high coding density when compared to polydnaviruses, at 70% it is quite low for a virus in general (Delaroque *et al.*, 2001), which could be indicative of some more intimate link between the genomes of the virus and its host. While the proposed replication cycle of EsV-1 is not as analogous to the polydnavirus replication cycle as Delaroque and Boland claim (2008), as the packaged EsV-1 virus is transmitted by lysis and infection of new algal cells rather than inherited vertically by the alga, it does illustrate how viruses may develop close interactions with the genomes of their hosts. Along similar lines, Bézier *et al.* (2009b) hypothesized based on nudivirus biology that the bracovirus ancestor may have been a sexually transmitted nudivirus which integrated into the wasp chromosome as part of its normal replication cycle.

Transposable elements provide an obvious potential mechanism for movement of genes between the genomes of virus and host. Indeed, extensive remnants of transposable elements have been identified in the genome of CcBV (Drezen *et al.*, 2006; Espagne *et al.*, 2004). Desjardins *et al.* (2008) identified a homologous Maverick-like transposable element in wasp DNA flanking the proviral DNA of GfBV, providing a simple scenario whereby transposable elements could move between flanking and proviral DNA. Furthermore, Desjardins *et al*. identified a p-element-like transposable element present in a small fraction of viral DNA of GiBV, suggesting that the insertion of the transposable element was recent and had not yet gone to fixation. These transposable elements not only provide a mechanism for moving genes between flanking and proviral DNA, but through a polydnavirus vector as a means of horizontal gene transfer between wasp and caterpillar host (Drezen *et al.*, 2006). It has already been shown that DNA from GiBV can be integrated *in vitro* into the genome of the wasp's lepidopteran host *Lymantria dispar* (Gundersen-Rindal and Dougherty, 2000).

In conclusion, while the genomes of Mimivirus and polydnaviruses share few homologous features, in parallel ways their unique elements have challenged our definition of viruses, and greatly expanded how we view the interactions of viruses with their hosts and the environment. The intimate relationship between parasitoid and virus, and amoeba and parasites, may provide ideal environments for close interaction of different genomes over an evolutionary time-scale. However, these systems may only represent a small set of environments predisposed to complex interactions between viruses, other parasites, and their hosts.

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