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Polintons: a hotbed of eukaryotic virus, transposon and plasmid evolution

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Abstract

Polintons (also known as Mavericks) are large DNA transposons that are widespread in the genomes of eukaryotes. We have recently shown that Polintons encode virus capsid proteins, which suggests that these transposons might form virions, at least under some conditions. In this Opinion article, we delineate the evolutionary relationships among bacterial tectiviruses, Polintons, adenoviruses, virophages, large and giant DNA viruses of eukaryotes of the proposed order ‘Megavirales’, and linear mitochondrial and cytoplasmic plasmids. We hypothesize that Polintons were the first group of eukaryotic double-stranded DNA viruses to evolve from bacteriophages and that they gave rise to most large DNA viruses of eukaryotes and various other selfish genetic elements.

All cellular life forms, with the possible exception of some extremely reduced intra-cellular parasites, are hosts to viruses and/or other selfish elements, such as transposons and plasmids[1]. This ‘greater virus world’ is enormously diverse in terms of replication mechanisms, gene-expression mechanisms, virion structure, and genome structure and size — viral genomes consist of single-stranded (ss) or double-stranded (ds) RNA or DNA and range in size from <2 kb to >2 Mb[2–4].

Viruses and other selfish elements do not share a single common ancestor; indeed, not a single gene is conserved in all or even most of these genomes[5,6]. However, selfish elements form a complex, dense network of evolutionary relationships in which genomes are linked through different shared genes[3]. This type of evolutionary relationship is most likely to have emerged from extensive gene exchange, sometimes between widely different elements.

Competing interests statement

The authors declare no competing interests.

FURTHER INFORMATION

Protein Data Bank: http://www.rcsb.org/pdb/home/home.do

SUPPLEMENTARY INFORMATION

See online article: S1 (figure) | S2 (figure) | S3 (figure) | S4 (table)

This article is dedicated to the memory of Jerzy Jurka (1950–2014), a co-discoverer of Polintons and a pioneer in the field of mobile genetic elements.

Viruses with large genomes possess many genes that have been acquired from the hosts at different stages of evolution. However, the hallmark genes of viruses, those that encode key proteins involved in genome replication and virion formation, are represented in a broad range of elements that form many edges in the evolutionary network[3,5,6].

The viruses present in archaea, bacteria and eukaryotes are fundamentally different[3,4]. In archaea and bacteria, most viruses possess dsDNA genomes. The second-most-common class includes small ssDNA viruses. Retrotranscribing elements comprise a small minority (no retroviruses are known) and RNA viruses are rare. Many dsDNA viruses as well as ssDNA viruses of archaea and bacteria alternate between lytic and temperate modes of reproduction. In the temperate mode, a viral genome integrates into a bacterial or archaeal chromosome and is transmitted vertically in the form of a provirus or prophage[4]; a typical bacterial or archaeal genome carries multiple prophages.

In contrast to bacteria and archaea, eukaryotes host numerous, diverse RNA viruses, retrotranscribing elements and retroviruses that typically integrate into the host genome[7,8]. In comparison with RNA viruses and retroelements, ssDNA and dsDNA viruses and mobile elements are less diverse and less common in eukaryotes, although both of these classes of selfish elements are widespread[3]. By far the largest group of DNA viruses in eukaryotes (BOX 1) consists of seven families of large and giant viruses (including mimiviruses and pandoraviruses, which have genomes in the megabase range). These families seem to share a common ancestry, as indicated by the conservation of approximately 50 (putative) ancestral genes[9–11]. Together, this group is known as the nucleocytoplasmic large DNA viruses (NCLDVs), or more recently the proposed order ‘Megavirales’ (REF. 12). The giant viruses of the family *Mimiviridae* are associated with a distinct class of satellite viruses, the virophages, which reproduce within viral ‘factories’ inside protist cells infected by the *Mimiviridae* and which depend on the latter for their replication[13–16].

Box 1

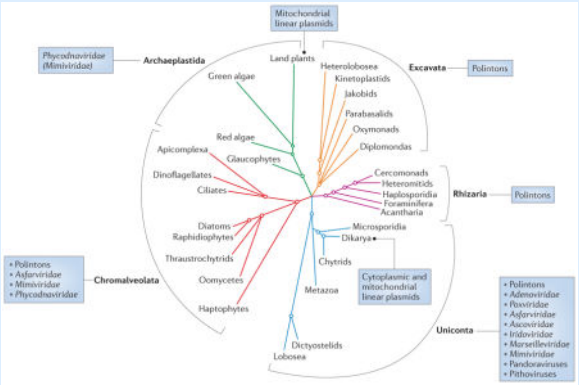
Distribution of Polintons, linear plasmids and ‘Megavirales’ in Eukarya

Viruses of the proposed order ‘Megavirales’ fall into seven well-established families[12], but some recently isolated Megavirales-derived viruses, such as pandoraviruses[71] and *Pithovirus sibericum*[69], remain unclassified. Thus far, Megavirales have been shown to propagate in organisms from three of the five eukaryotic supergroups[72], namely Archaeplastida, Chromalveolata and Uniconta (see the figure). The Megavirales associated with the Uniconta show the greatest diversity: within this supergroup, members of the families *Marseilleviridae* and *Mimiviridae*, as well as unclassified pandoraviruses and pithovirus, infect amoebae; members of the *Ascoviridae* infect insects; iridoviruses are associated with either insects or fish; poxviruses infect various animals, including insects, reptiles, molluscs, birds and mammals; and *Asfarviridae* infect mammals. In the Chromalveolata, members of the *Phycodnaviridae* infect brown algae (phylum Heterokontophyta) and coccolithophores (phylum Haptophyta); some members of the *Mimiviridae* prey on microflagellate grazers (Heterokontophyta) and bloom-forming microalgae (Haptophyta); and a putative member of *Asfarviridae* infects

dinoflagellates. Finally, phycodnaviruses and some mimiviruses are known and thought, respectively, to infect green algae in the eukaryotic kingdom Archaeplastida.

Polintons and related large DNA transposons have an equally wide, if not wider, distribution in Eukarya[20,21]. Polintons are widespread in Uniconta, where they are found in diverse Amoebozoa, Metazoa and Dikarya (fungi). Among the Chromalveolata, Polintons are present in oomycetes, whereas a distinct type of Polinton, known as Tlr1 (Polinton-like transposable element from *Tetrahymena thermophila*) elements, is found in ciliates (phylum Ciliophora). Polintons have also been described in Excavata, in which they are extremely abundant in the parabasalid *Trichomonas vaginalis*, comprising up to 30% of the genome[21]. Polintons have not been previously described in Rhizaria. However, BLASTP (protein–protein BLAST) searches seeded with Polinton proteins showed that the partially sequenced genome of the foraminiferan *Reticulomyxa filosa* contains genes for key Polinton proteins, including protein-primed type B DNA polymerase, A32-like genome-packaging ATPase and RVE family integrase (Supplementary information S4 (table)). Thus, of the five major eukaryal kingdoms, only one (that is, Archaeplastida) does not contain identifiable Polintons.

In contrast to the wide distribution of Megavirales and Polintons, mitochondrial linear plasmids are found only in filamentous fungi (Dikarya) and plants (Viridiplantae), and so far the cytoplasmic plasmids are restricted to yeast (Dikarya)[46,48].



Recently, an evolutionary connection has been shown to exist between the virophages and large eukaryotic dsDNA transposons of the Polinton family (also known as the Maverick family)[17,18]. The Polintons are widely distributed in diverse protists and animals (BOX 1), indicative of their ancient origin (which is perhaps concomitant with the origin of eukaryotes) and evolutionary success. We have recently shown that most Polintons encode two proteins that are homologous to viral capsid proteins, suggesting that at least under some conditions, Polintons can produce virions that could infect new hosts[19]. Thus, Polintons, which would perhaps be more properly named polintoviruses, seem to combine features of viruses and transposons. However, until production of viral particles is experimentally verified, we continue to refer to these virus-like elements as Polintons.

These findings prompted us to revisit the evolutionary connections between the Polintons and other viruses and mobile elements of archaea, bacteria and eukaryotes. Here, we provide

evidence to support an evolutionary scenario under which Polintons evolved directly from bacteriophages and became an evolutionary hotbed that gave rise to most of the large dsDNA viruses of eukaryotes, in addition to several groups of plasmids and transposons.

Conserved genome architecture

The Polinton genomes are 15–20 kb in size and encode several conserved proteins, including protein-primed type B DNA polymerase (pPolB), retroviral-like (RVE) family integrase, FtsK-like ATPase, adenovirus-type cysteine protease[20–22] and two putative capsid proteins[19]. Polintons contain terminal inverted repeats (TIRs) and are thought to replicate by a protein-primed mechanism involving the element-encoded pPolBs. In some groups of Polintons, the putative capsid-protein-encoding genes have apparently been lost (BOX 1). Polintons from distant organisms display substantial variation in genome organization and gene content[18,22]. In addition to the conserved gene set outlined above, different Polintons encode less-conserved proteins, such as various helicases and primases, with homologues in viruses and transposons (BOX 2).

Box 2

Proteins shared between Polintons and ‘Megavirales’

In addition to the highly conserved proteins involved in virion morphogenesis, at least 13 protein families encoded by different representatives of the proposed order ‘Megavirales’ have homologues in Polintons (see the table). Of particular note are the shared proteins that are potentially involved in genome replication and transcription. For example, Polintons from *Glomus intraradices* and *Trichomonas vaginalis* encode a divergent D5-like helicase–primase that is one of the hallmarks of the Megavirales. A Polinton of *Dictyostelium fasciculatum* encodes a superfamily 3 helicase, whereas elements of *Tetrahymena thermophila* and *Hydra magnipapillata* contain genes for PIF1-like superfamily 1 helicases. A related PIF1-like helicase is also encoded by transpovirons (FIG. 1), a group of recently described linear plasmids that replicate in association with mimiviruses[73]. Another element of *H. magnipapillata* encodes a dUTPase, an enzyme often found in Megavirales genomes[10]. A Polinton from *Nematostella vectensis* encodes a transcription initiation factor, TFIIB, that closely matches the corresponding proteins from marseilleviruses.

Some of the genes shared by Megavirales and Polintons might have been independently acquired by the respective viruses from distinct sources, as has been demonstrated for many of the Megavirales genes[66]. Nevertheless, it seems to be most likely that the core structural and morphogenetic proteins of the Megavirales — including two capsid proteins, the packaging ATPase and the maturation protease — have been derived from the Polinton ancestor.

NCVOG	Protein*	Distribution among Polintons or plasmids	Best-match expectation (E) value
Megavirales proteins highly conserved in Polintons			

NCVOG	Protein*	Distribution among Polintons or plasmids	Best-match expectation (E) value
NCVOG0022	Double jelly-roll major capsid protein	Nearly universal	Structural modelling
NCVOG0249	A32-like packaging ATPase	Nearly universal	2.65E-09
NCVOG0246	Ulp1 protease (PF02902)	Nearly universal	2.05E-06
NCVOG0584	Putative penton (mimiviruses and phycodnaviruses)	Nearly universal	3.58E-03
Megavirales proteins sporadically found in Polintons			
NCVOG0045	Highly derived D5-like helicase–primase	TV, GI and Df (S3H domain)	1.02E-30
NCVOG0248	PIF1-like ATP-dependent DNA helicase (PF05970)	HM and Tt	9.10E-43
NCVOG1068	dUTPase (PF00692)	HM	1.20E-24
NCVOG1127	Transcription initiation factor TFIIB (PF00382)	NV	3.27E-17
NCVOG1086	259R of invertebrate iridescent virus 6	NV and HM	8.37E-09
NCVOG0009	BIR domain (PF00653)	TC	1.81E-12
NCVOG1360	KilA domain (PF04383)	TV	3.76E-12
NCVOG0933	MSV079 of *Melanoplus sanguinipes* entomopoxvirus	HM, TC, Nvi, NV, DBi, DGr and AP	1.95E-08
NCVOG0965	Protein phosphatase 1 (PF10488)	TC, Nvi and Gf	1.49E-07
NCVOG1349	Hypothetical *Chlorovirus* and *Ostreococcus* virus protein	Tt	4.75E-17
NCVOG0072	HNH endonucleases (PF01844), found in marseille-, mimi- and phycodnaviruses	Tt and TV	1.68E-09
NCVOG0010	Bro-N (PF02498)	NV	9.19E-19
No NCVOG	pB263R of African swine fever virus	GI	5.49E-22
Megavirales proteins highly conserved in cytoplasmic plasmids			
NCVOG0031	D6/D11 helicase (DEAD/SNF2-like helicases)	Universally present	1.26E-31
NCVOG0271	DNA-dependent RNA polymerase, β-subunit	Universally present	7.34E-23
NCVOG1117	mRNA-capping enzyme, large subunit	Universally present	4.11E-05

*When available, protein family (PF) identifiers from the Pfam database are indicated. AP, *Acyrtosiphon pisum*; DBi, *Drosophila biarmipes*; Df, *Dictyostelium fasciculatum*; DGr, *Drosophila grimshawi*; Gf, *Glyptapanteles flavicoxis*; GI, *Glomus intraradices*; HM, *Hydra magnipapillata*; NCVOG, nucleocytoplasmic virus orthologous group; NV, *Nematostella vectensis*; Nvi, *Nasonia vitripennis*; TC, *Tribolium castaneum*; Tt, *Tetrahymena thermophila*; TV, *Trichomonas vaginalis*.

FIGURE 1 shows the homologous genes and gene blocks that are shared between the Polintons and other viruses, transposons and plasmids. All of these elements form a network of connections in which the edges are homologous genes (FIG. 1a). Polintons share the

largest number of genes with mobile elements from the other nodes and hence represent the central hub of the network (FIG. 1a). Most notable are the multiple connections between bacteriophages of the family *Tectiviridae*, Polintons and the Mavirus virophage. All of these elements share four genes that encode two capsid proteins, a DNA-packaging ATPase and pPolB (FIG. 1b) (see below). Polintons share two additional genes with the Mavirus virophage, namely those for the capsid-maturation protease and the RVE integrase. The other known virophages lack pPolB and RVE integrase but share the capsid proteins, ATPase and protease. The adenoviruses join the network (FIG. 1) through pPolB, the two capsid proteins and the protease, whereas the much larger Megavirales possess the capsid proteins, the ATPase and the protease. The linear cytoplasmic plasmids isolated from yeast form a bridge between the mobile elements with protein-primed replication and the much more complex Megavirales; these plasmids encode pPolB along with four key proteins that are conserved in most of the Megavirales (FIG. 1). Below, we examine these homologous relationships in greater detail before proposing an evolutionary scenario in which diverse eukaryotic viruses and other mobile elements are derived from the Polintons.

Virion morphogenesis proteins

Most Polintons encode two putative capsid proteins, one of which is predicted to adopt the double jelly-roll (DJR) topology[19] found in the major capsid proteins (MCPs) of diverse dsDNA viruses that infect bacteria, archaea and eukaryotes[23–25]. The other predicted capsid protein has a single jelly-roll fold and corresponds to the minor capsid protein (mCP), often called the penton protein, which is also conserved in viruses with DJR MCPs[26–30]. In principle, the two proteins — the MCP and the mCP — are sufficient (and necessary) to construct the entire icosahedral capsid (FIG. 2), although viruses often encode additional minor structural proteins. The conservation of the MCP and mCP in Polintons closely follows the presence of the genome-packaging ATPase and the cysteine protease (that is, all of these proteins are either present or absent), which are also predicted to be involved in virion morphogenesis[19].

Major capsid proteins

As discussed above, Polinton genomes have a similar layout to those of bacterial tectiviruses, eukaryotic adenoviruses and virophages (FIG. 1), suggesting a specific evolutionary connection among all of these elements. However, the MCP of Polintons is most closely related to the MCPs of phycodnaviruses[19], which are members of the proposed order Megavirales[12]. FIGURE 2 illustrates the similarity between the more compact MCPs of Polintons and the phycodnavirus *Paramecium bursaria Chlorella* virus 1 (PBCV1), and highlights the differences between these proteins and the larger MCPs of the Sputnik virophage and adenoviruses. The evolutionary scenario underlying this relationship remains uncertain, but it might involve fast evolution of the MCP in the virophages and adenoviruses. Thus, the presence of phycodnavirus-like MCPs in Polintons establishes an evolutionary link between the giant Megavirales and dsDNA viruses with smaller genomes.

Minor capsid proteins

The mCPs of Polintons correspond to the penton proteins that are found at the fivefold vertices of icosahedral capsids (FIG. 2). Penton proteins have been observed in all viruses with DJR MCPs for which high-resolution structural information is available, including tectivirus PRD1 (REF. 26), corticovirus PM2 (REF. 27), archaeal turrivirus STIV (*Sulfolobus* turreted icosahedral virus)[28], virophage Sputnik[29] and adenoviruses[30]. Thus far, an equivalent of the penton protein has not been identified among the Megavirales; however, considering the importance of this protein for the formation of icosahedral virions, one could expect that these giant viruses also encode a penton homologue[31]. Using the predicted mCP of Tlr1 (Polinton-like transposable element from *Tetrahymena thermophila*) as a seed in PSI-BLAST (position-specific iterative basic local alignment search tool) sequence similarity searches, we detected penton-like proteins in mimiviruses and phycodnaviruses (Supplementary information S1 (figure)). Consistent with this, in PBCV1 the predicted penton (A533R) has been detected in the virion[32]. In mimiviruses, the predicted penton-protein gene is adjacent to the gene encoding the maturation protease (FIG. 1), an arrangement that is also found in Mavirus and some Polintons. The finding that not only the MCP but also the mCP of Polintons has counterparts in Megavirales further strengthens the link between these genetic elements.

Cysteine proteases

Polintons encode a conserved cysteine protease that is homologous to the adenovirus maturation protease and eukaryotic Ulp1-like proteases, both classified as members of the CE (cysteine endopeptidase) clan of proteases[33]. In adenoviruses, the protease is the principal player responsible for transforming immature virions into fully infectious, mature virus particles[34]. In addition to adenoviruses, homologous proteases are encoded by virophages and most of the Megavirales[18,35]. In adenoviruses, poxviruses and asfarviruses, the proteases are responsible for proteolytic processing of immature virions[36,37]. The wide distribution and importance of these proteases in the maturation of both small and large eukaryotic viruses with DJR MCPs suggests that acquisition of the protease antedates the radiation of these viruses. By contrast, none of the viruses in archaea and bacteria with a DJR MCP encodes, or is known to require, a protease for virion maturation. Indeed, the key difference between Polintons and bacterial tectiviruses is that the latter lack genes for the cysteine protease and RVE family integrase. Although some tectiviruses form plasmidial prophages, none of these viruses has been found to integrate into the host genome[38,39]. Notably, some eukaryotic transposons carry genes for both integrase and cysteine protease[22]. For example, the DNA transposons of the Ginger 1 family encode Polinton-like RVE integrases containing carboxy-terminal Ulp1-like cysteine protease domains[40] (FIG. 1). Thus, the proteases and integrases could have been incorporated into an ancestral viral genome through the integration and subsequent domestication of a DNA transposon.

Genome-packaging ATPases

All icosahedral viruses with DJR MCPs in archaea, bacteria and eukaryotes, with the exception of adenoviruses, encode genome-packaging ATPases of the FtsK/HerA superfamily[41,42]. The vaccinia virus protein A32 and the P9 protein of tectivirus PRD1 are

essential for pumping the respective viral genomes into preformed immature virions[42,43]. However, adenoviruses encode a distinct ATPase (IVa2) that has the same role[34,44]. Although most Polintons encode A32-like ATPases[19], some contain a gene encoding an ATPase that is more closely related to adenovirus IVa2 (Supplementary information S2 (figure)). Given the greater genomic diversity and broader taxonomic distribution of Polintons compared with adenoviruses, which are restricted to Metazoa (BOX 1), the diversification of packaging ATPases probably occurred during the evolution of Polintons, and IVa2 was subsequently inherited by adenoviruses from a specific Polinton lineage. This directionality is consistent with the phylogenetic analysis of the pPolBs, as described below.

Protein-priming mobile elements

Protein-primed DNA replication is an exclusive feature of viruses and plasmids that encode pPolBs. To initiate genome replication, these elements use a terminal protein that remains covalently attached to the 5′ termini of their linear genomes instead of the nucleic-acid primers that are used by cellular organisms and most DNA viruses. Terminal proteins are often encoded by separate genes[45], but in some plasmids they are fused to the amino termini of their cognate pPolBs[46]. A similar fusion has been detected in Polintons and bidnaviruses[47] (FIG. 1).

FIGURE 3 shows a phylogenetic tree of pPolBs from a wide range of viruses and plasmids in archaea, bacteria and eukaryotes. In this phylogeny, pPolBs are segregated into two major groups: bacterial and archaeal mobile elements, and eukaryotic plasmids and viruses. Among the pPolB-encoding elements in archaea and bacteria, only tectiviruses share additional genes with the eukaryotic pPolB-encoding viruses and plasmids (FIG. 1).

The pPolBs from eukaryotic mobile elements form two clades. One clade exclusively contains linear plasmids that replicate in the mitochondria of filamentous fungi and some plants[46,48]. All of these plasmids contain TIRs and commonly encode two proteins: a pPolB fused to the terminal protein and a single-subunit DNA-dependent RNA polymerase (RNAP) related to the mitochondrial RNAPs that are encoded in the nucleus and derive from T7-like bacteriophages[49,50]. The second eukaryotic pPolB clade includes cytoplasmic linear plasmids from yeast and all eukaryotic viruses that use protein priming (FIG. 3). Notably, Polintons are at the base of this group, whereas cytoplasmic plasmids, adenoviruses and bidnaviruses emerge from within the Polinton clade (FIG. 3).

Adenoviruses

All known adenoviruses infect vertebrates and possess capsids that are geometrically identical to those of tectiviruses; that is, the number and arrangement of hexagonal capsomers (DJR MCP trimers) and pentagonal capsomers (penton pentamers) is exactly the same in these two groups of viruses[26,51] (FIG. 2). Despite the strong evidence of the evolutionary relationship between tectiviruses and adenoviruses[23,52,53], the exact evolutionary trajectory linking them has remained obscure. In our phylogenetic analysis, viruses of the four major genera of the family *Adenoviridae* form a well-supported clade that emerges from within Polintons (FIG. 3), in agreement with previous findings[18]. Given the comparatively low divergence of known adenoviruses and lack of known representatives

outside vertebrates[52], we suggest that adenoviruses probably evolved from Polintons relatively late in eukaryotic evolution.

Bidnaviruses

Members of the recently established family *Bidnaviridae* are small ssDNA viruses that infect insect hosts[54]. They have bipartite linear genomes with TIRs and, unlike other ssDNA viruses, encode pPolBs[55]. A recent analysis of the complex evolutionary history of bidnaviruses has shown that the ancestor of this viral group evolved from an arthropod-infecting ssDNA virus of the *Parvoviridae* family via multiple gene exchanges with diverse RNA and DNA viruses[47]. A key point in bidnavirus evolution was the loss of the rolling-circle replication initiation protein gene and acquisition of the pPolB-encoding gene from an insect Polinton.

Virophages

Mavirus-like virophages represent the fourth group of eukaryotic pPolB-encoding viruses[17]. Although virophages were excluded from the phylogenetic tree in FIG. 3 owing to the high divergence of their pPolBs, resulting in distortion to the tree topology, separate analyses place the virophages inside the Polinton branch of pPolB[22] (Supplementary information S3 (figure)). Multiple lines of evidence have recently indicated that the genes shared between virophages and Polintons originated during the evolution of Polintons[18], in contrast to the originally proposed scenario in which Polintons were derived from virophages[17]. In our view, the identification of Polinton DJR MCPs and mCPs leaves little doubt that virophages are direct descendants of Polintons[19]. Virophages show notable variation in gene content. In Sputnik-like virophages, the pPolB gene has been replaced with a gene encoding a distinct polymerase primase[18,56]. Although we remained uncertain about the genomic layout of the ancestral virophage in our previous analysis[18], the evolutionary relationship between Polintons and virophages seems to point to a Mavirus-like virophage as the most likely ancestral state.

Cytoplasmic plasmids

A group of linear plasmids, distinct from those found in mitochondria of filamentous fungi and plants, is present in various yeast species[46,57]. These plasmids reside exclusively in the cytoplasm of their hosts and encode key components of their own replication and transcription machineries (FIG. 1). In phylogeny, pPolBs of the cytoplasmic plasmids emerge from within the Polintons as a sister group to adenoviruses and show no affinity to the mitochondrial plasmids (FIG. 3). As in Polintons, bidnaviruses and mitochondrial plasmids, the terminal proteins of linear cytoplasmic plasmids are fused to the N termini of the pPolBs[46,47], suggesting that these plasmids share the most recent common ancestor with Polintons rather than adenoviruses. These plasmids do not encode any viral structural proteins that could have been lost during evolution. Notably, certain linear bacterial plasmids (for example, pBClin15) evolved from tectiviruses[39] (FIG. 3), suggesting that a similar evolutionary transition could have occurred in the case of Polintons.

The RNAPs encoded by the cytoplasmic plasmids are unrelated to the single-subunit RNAPs of mitochondrial plasmids; instead, they are homologous to the two largest subunits (the β-

and β′-subunits) of multisubunit RNAPs encoded by cellular organisms and members of the Megavirales[10,46,57,58]. The plasmid RNAPs are encoded by two genes (FIG. 1); the larger one encodes a subunit containing all characteristic motifs of the β-subunit and some of the motifs of the β′-subunit, whereas the second, shorter gene encodes a protein bearing additional motifs of the β′-subunit[57,59]. The cytoplasmic plasmids encode two additional proteins, the D11-like helicase and the mRNA-capping enzyme, which show specific evolutionary relationship to the homologues from Megavirales (FIG. 1) and are likely to be essential for the cytoplasmic replication of DNA genomes in eukaryotes, given the ubiquity of the respective genes in all known cytoplasmic DNA elements. In vaccinia virus (*Poxviridae*), the D11 protein (a DExH helicase family member) has an important role during transcription elongation and mRNA release on termination[60]. The mRNA-capping enzymes encoded by cytoplasmic plasmids possess a unique domain architecture that is not found in cellular organisms but only in diverse members of the Megavirales[61–63]. These viral and plasmid enzymes consist of three functional domains, namely RNA 5′-triphosphatase, RNA guanylyltransferase and RNA (guanine-N7)-methyltransferase, which in a series of consecutive reactions synthesize the 7-methylguanosine RNA cap on the nascent transcripts[62–65].

Based on the unique shared domain architecture of the capping enzymes, Shuman has proposed that poxviruses and asfarviruses evolved from linear cytoplasmic plasmids[63]. However, neither the origin of the cytoplasmic plasmids themselves nor the source of the virion morphogenetic unit of the Megavirales has been clarified. By contrast, Klassen and Meinhardt have proposed that eukaryotic linear plasmids evolved from an adenovirus-like or tectivirus-like ancestor through the loss of genes required for virion formation[46]. Inclusion of the Polintons provides a better-supported scenario for the origin of both linear plasmids and Megavirales. Indeed, the pPolB phylogeny implies that cytoplasmic plasmids evolved from a Polinton ancestor rather than from a tectivirus or an adenovirus (FIG. 3). We hypothesize that a Polinton escaped from the nucleus by acquiring the RNAP, capping apparatus and D11-like helicase from the host[66], and subsequently followed two opposite evolutionary pathways, one leading to the origin of cytoplasmic plasmids and the other to the emergence of the ancestor of Megavirales (see below).

Polintons: a hotbed of virus evolution

The comparative analysis of the various viruses, plasmids and transposons in archaea, bacteria and eukaryotes presented here places the Polintons at the root of many families of eukaryotic DNA viruses. Integrating different lines of evidence, we propose a unifying scenario in which Polintons were a hotbed of eukaryotic virus, transposon and plasmid evolution (FIG. 4). In this scenario, Polintons evolved at the onset of eukaryogenesis from a tectivirus-like ancestor. This virus could have entered the proto-eukaryotic host along with the bacterial endosymbiont that subsequently gave rise to the mitochondria (FIG. 4). This route of evolution is consistent with the presence of linear plasmids in the mitochondria of fungi and plants and the primary split between the pPolBs of mitochondrial plasmids and the rest of the eukaryotic plasmids and viruses (FIG. 3).

The major distinction between tectiviruses and Polintons is the presence in the latter of the genes encoding the Ulp1-like cysteine protease and RVE family integrase. Both of these genes could have been introduced into the genome of the tectiviral ancestor of the Polintons in a single recombination event with a eukaryotic DNA transposon. Notably, Ulp1-like cysteine proteases are characteristic of eukaryotes, whereas bacteria encode only distantly related cysteine proteases, suggesting that the Polinton ancestor had already acquired the protease and integrase genes in the (proto-)eukaryotic host. The protease was adopted for virion maturation and retained in all major virus lineages emerging from Polintons, including virophages, adenoviruses and Megavirales, although it was subsequently lost from some members of the Megavirales.

The acquisition of the RVE integrase gene granted the emerging Polintons the ability to follow two alternative lifestyles, one that is typical of transposable elements and one that is typical of bona fide viruses. Such duality is also embraced by Mu-like bacteriophages and eukaryotic metaviruses (Ty3-gypsy retrotransposons), and pseudoviruses (Ty1-copia retrotransposons)[3,4,67]. The ability to persist in the integrated form in the host genome could have played a key part in the further diversification and successful spread of Polintons and their derivatives in eukaryotes. Integration into the host chromosome provides a ‘safe haven’ for viral genomes, where they have the opportunity to accumulate changes by genetic drift or recombination without the immediacy of producing infectious virus progeny. After a viable and sufficiently fit individual evolves, it can ‘spring out’ of the host chromosome and revert to the viral way of life.

Some Polintons seem to have abandoned the virus-like reproduction strategy altogether and have lost the genes implicated in virion formation[19]. The loss of the genes for DJR MCPs might be linked to the extraordinary expansion of Polintons in *Trichomonas vaginalis*, where they constitute up to 30% of the host genome[21]. The ancestor of adenoviruses seems to have followed the opposite evolutionary route, whereby the integrase gene was lost along with the transposition ability (FIG. 4), thus committing adenoviruses to the strict viral strategy. Polintons are also implicated in the evolution of ssDNA viruses of the *Bidnaviridae* family; this family emerged as a result of extensive gene shuffle between four groups of selfish elements, including Polintons, which contributed pPolB[47].

The major legacy of Polintons is the central role they are likely to have had in the emergence of the Megavirales, the most widespread and diverse group of eukaryotic dsDNA viruses. The Megavirales apparently inherited from the Polintons the key proteins involved in virion morphogenesis, including the DJR MCP, the genome-packaging ATPase, the maturation protease and possibly the penton protein (FIG. 1). The Polinton MCP is more closely related to those of phycodnaviruses than it is to the MCPs of any other viral groups, including tectiviruses[19] (FIG. 2), which is suggestive of an evolutionary link between Polintons and Megavirales. Although the packaging ATPases and maturation proteases both show high levels of sequence divergence, the topologies of the respective phylogenetic trees are compatible with the existence of such a link[18].

Polintons reside in the nucleus and accordingly rely on host enzymes for transcription. The major event underlying the emergence of the Megavirales was apparently the escape from

the nucleus, which was associated with the acquisition of RNAP and the capping apparatus from the host. The putative escaped element that would replicate in the cytoplasm using the ancestral Polinton pPolB gave rise to two groups of mobile elements (FIG. 4); namely, the cytoplasmic plasmids and Megavirales that share the unique trifunctional capping enzyme, RNAP and D11-like helicase (FIG. 1). The cytoplasmic plasmids retained pPolB but lost the genes implicated in virion morphogenesis, succumbing to the exclusive intra-cellular lifestyle. By contrast, the ancestor of the Megavirales evolved via the route of increasing complexity.

The essential events in the evolution of Megavirales from the cytoplasmic Polinton-like ancestor were the replacement of the pPolB with the RNA/DNA-primed PolB and the acquisition of the D5-like helicase–primase (FIG. 4). The pPolBs might be unable to ensure efficient replication of genomes larger than a certain threshold size (~45 kb, as in adenoviruses), conceivably owing to the lack of a dedicated primase that would provide multiple internal primers along the genome. Notably, some Polintons encode divergent D5-like helicase–primases (FIG. 1; BOX 2), which tend to cluster with the homologues from Megavirales in phylogenetic trees[18]. This suggests that Megavirales inherited this key enzyme from Polintons. Several other genes that are common and probably ancestral in the Megavirales are also shared with various Polintons (BOX 2). The PolB of Megavirales was probably acquired from the eukaryotic host[66], replacing the ancestral pPolB and, together with the helicase–primase, opening the route to genome expansion. The extent of this expansion, which involved massive acquisition of genes not only from eukaryotes but also from bacteria[68], was such that the genes apparently inherited from Polintons comprise but a tiny proportion of the gene complement of these large and giant viruses. Crucially, however, a few losses notwithstanding, the proteins of Polinton descent form the core of the virion morphogenesis machinery, as well as important parts of the replication apparatus, in most members of the Megavirales (BOX 2).

The evolution of the Megavirales was marked by genome expansion that has been pushed to the extreme in at least three independently evolved groups of giant viruses: *Mimiviridae*, which are distantly related to *Phycodnaviridae*; pandoraviruses, which evolved from a common ancestor with coccolithoviruses within the *Phycodnaviridae*; and pithoviruses, which are related to *Iridoviridae* and *Marseilleviridae*[68,69]. The ancestral icosahedral capsids constructed from DJR MCPs were substituted with less-regular, ovoid or brick-shaped virion morphologies in several groups within the Megavirales — namely, in ascoviruses, poxviruses, pandoraviruses and pithoviruses — indicating that all facets of the viral life cycle are susceptible to dramatic changes. Unlike in the case of adenoviruses, the wide distribution of Megavirales in eukaryotes (BOX 1) implies that these viruses diverged from the Polinton ancestor early in eukaryal evolution.

The virophages followed a different strategy to escape from the nucleus. Instead of encoding their own factors that would enable their cytoplasmic propagation, these viruses evolved to parasitize on their giant relatives by hijacking the necessary molecular machinery[14–17].

Conclusions

The extended virus world, which includes both bona fide viruses and related mobile elements, is a complex network of genomes that share partially overlapping sets of genes[3]. Discerning specific evolutionary scenarios in this maze is a major challenge. In particular, the origins of the diverse groups of eukaryotic viruses remain obscure. Clearly, the evolution of these viruses involved contributions from bacteriophages, as particularly indicated by the relationships between the respective capsid proteins and packaging enzymes[25,41,70]. It is equally clear that some of the key viral genes were acquired from the hosts at different stages of evolution, but otherwise the routes of viral evolution remain hazy. The synthesis of phylogenomic analyses presented here pushes the evolutionary study of eukaryotic DNA viruses beyond general considerations and into the territory of concrete, evidence-based reconstruction. The central role in our emerging scenario belongs to the Polintons, a remarkable group of selfish elements that are nearly ubiquitous in eukaryotes and combine features of integrating mobile elements with those of bona fide viruses. Polintons show strong evolutionary connections with bacteriophages of the family *Tectiviridae*, suggesting that Polintons were the first group of eukaryotic dsDNA viruses to evolve from archaeal and bacterial ancestors. The apparent dual lifestyle of the Polintons seems to have entailed outstanding evolutionary resilience and versatility, making them a hotbed of evolution of eukaryotic selfish elements that spawned diverse groups of viruses in a broad range of sizes, along with related plasmids. This evolutionary scenario parallels the apparent course of evolution of retrotranscribing viruses from retroelements with a dual lifestyle[3]. In even more general terms, the emerging story of virus evolution is compatible with the concept of viral hallmark genes that form different combinations in diverse selfish elements and on multiple occasions serve as ‘kernels’ for gene accretion and genome expansion.

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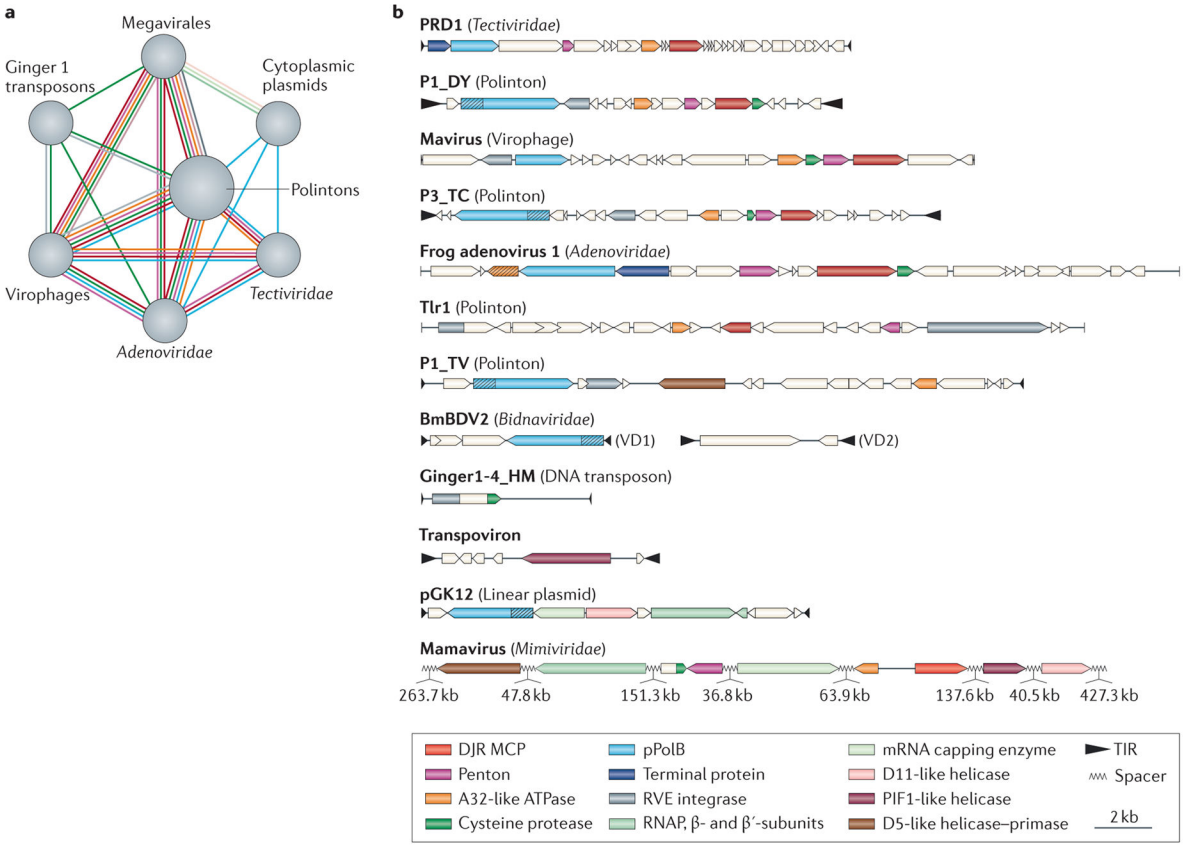

Figure 1. Evolutionary relationships between Polintons and other mobile genetic elements

a | Evolutionary network showing shared gene content between Polintons and other mobile genetic elements in archaea, bacteria and eukaryotes. Edges correspond to homologous genes. The colour key is provided in part **b**. Only those elements that are directly linked to Polintons are shown. **b** | Genome maps of various viruses, plasmids and transposons in archaea, bacteria and eukaryotes. Homologous genes are colour-coded. Hatched regions in the protein-primed type B DNA polymerase (pPolB) genes indicate the position of the (predicted) terminal protein domains. Hatching is also used to indicate the gene encoding the distinct adenoviral genome packaging ATPase IVa2. VD1 and VD2 are the two genomic segments of the *Bombyx mori* bidensovirus 2 (BmBDV2). DJR, double jelly-roll; Ginger1-4_HM, transposon 4 of *Hydra magnipapillata*; MCP, major capsid protein; P1_DY, Polinton 1 of *Drosophila yakuba*; P1_TV, Polinton 1 of *Trichomonas vaginalis*; P3_TC, Polinton 3 of *Tribolium castaneum*; RNAP, RNA polymerase; TIR, terminal inverted repeat; Tlr1, Polinton-like transposable element from *Tetrahymena thermophila*.

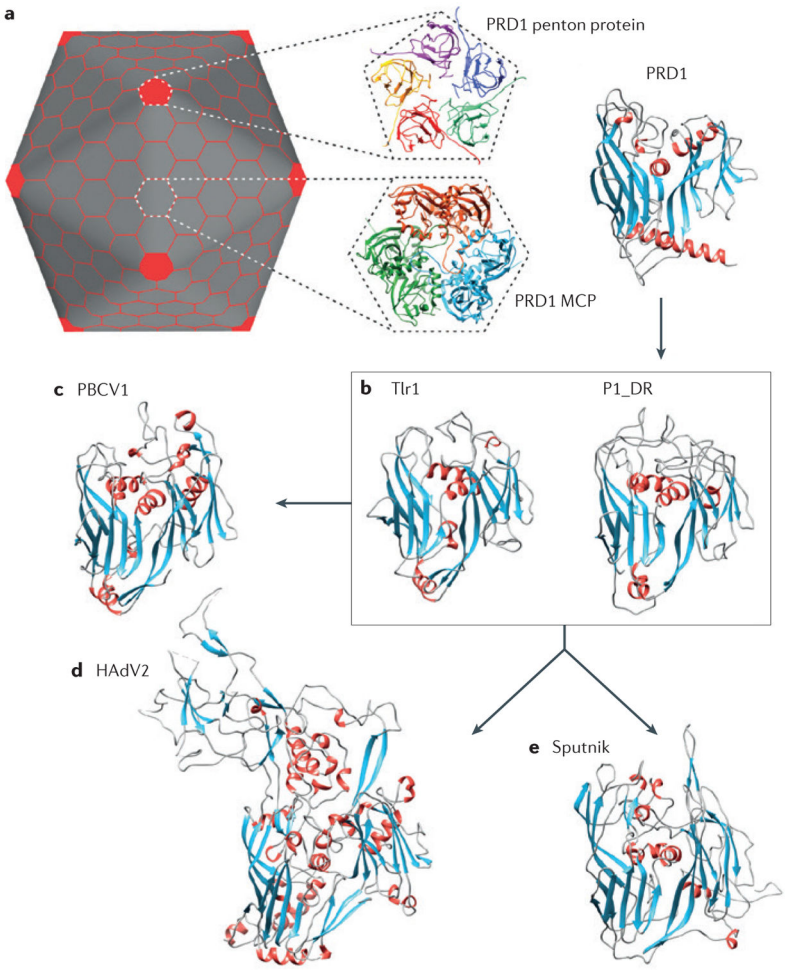

Figure 2. Structural features of some viruses with double jelly-roll major capsid proteins

a | Representation of the icosahedral virion with triangulation number (T)=25; this organization is found in tectiviruses and adenoviruses. The pentagonal capsomers (shown in red) are composed of five copies of the penton protein, which has a single jelly-roll fold, whereas the hexagonal capsomers are trimers of the major capsid protein (MCP) with the double jelly-roll (DJR) topology. X-ray structures of the tectivirus PRD1 penton and MCP are shown as examples (Protein Data Bank (PDB) identifier: 1W8X)[26]. **b** | Structural models of the MCPs of Tlr1 (Polinton-like transposable element from *Tetrahymena thermophila*) and P1_DR (Polinton 1 from *Danio rerio*)[19]. **c–e** | X-ray structures of the DJR MCPs from the eukaryotic viruses PBCV1 (*Paramecium bursaria Chlorella* virus 1; PDB identifier: 1M4X)[74] (**c**), HAdV2 (human adenovirus type 2; PDB identifier: 1P2Z)[75] (**d**) and Sputnik (PDB identifier: 3J26)[29] (**e**).

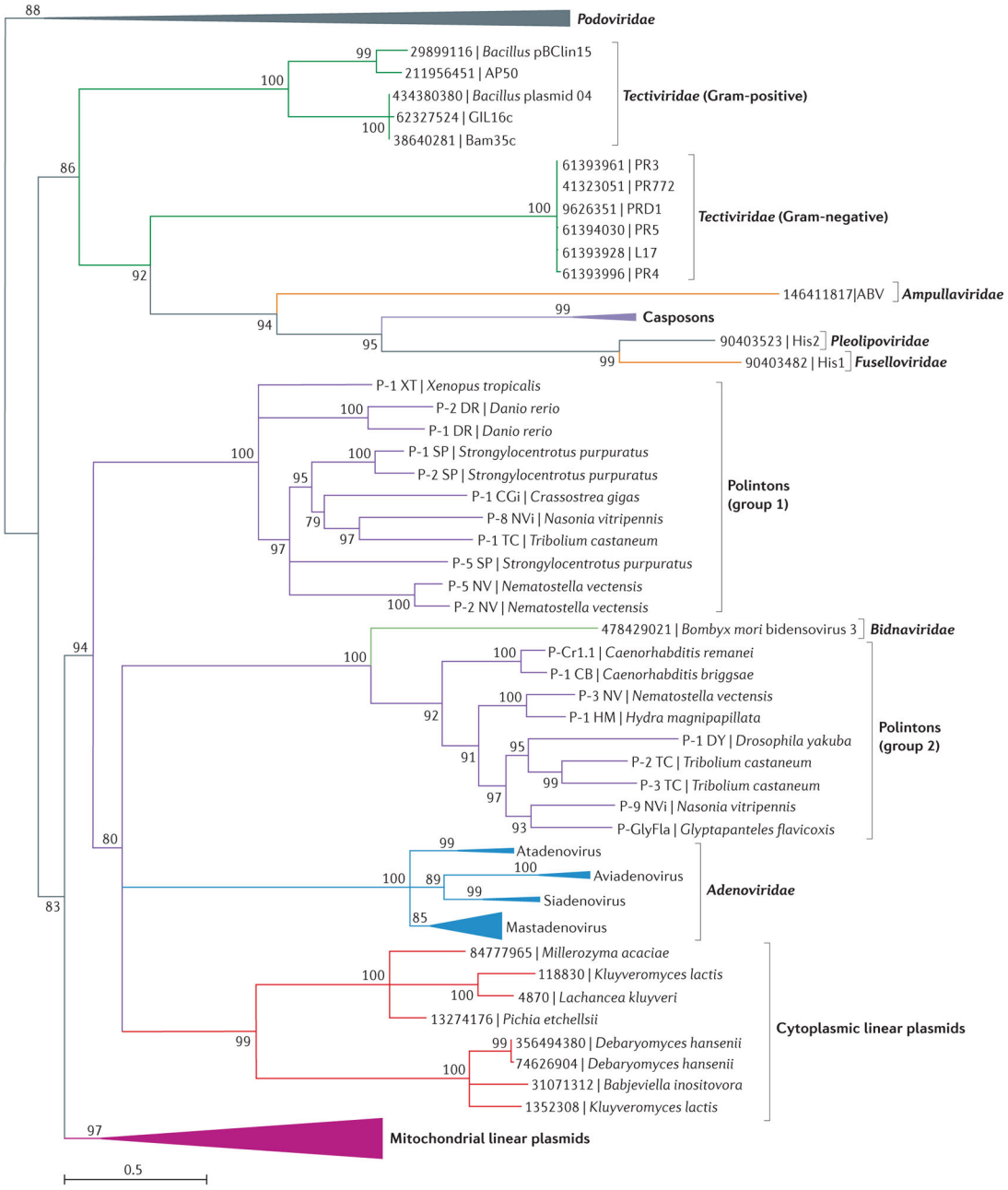

Figure 3. Phylogenetic analysis of protein-primed type B DNA polymerases from mobile elements in archaea, bacteria and eukaryotes

The maximum likelihood tree was calculated using PhyML[76], with the WAG (Whelan and Goldman) model of amino-acid substitution, including a gamma law (four categories) and an estimated proportion of invariable sites. Numbers at the branch points represent SH (Shimodaira–Hasegawa)-like local support values. The tree is rooted with bacterial phi29-like podoviruses. Branches with support values below 75% were collapsed. Branches are coloured according to the classification of the corresponding taxa. The branching of the major taxonomic groups is consistent with that obtained previously using different

phylogenetic analysis methods and different taxonomic sampling[20,46,47,77]. The scale bar represents the number of substitutions per site.

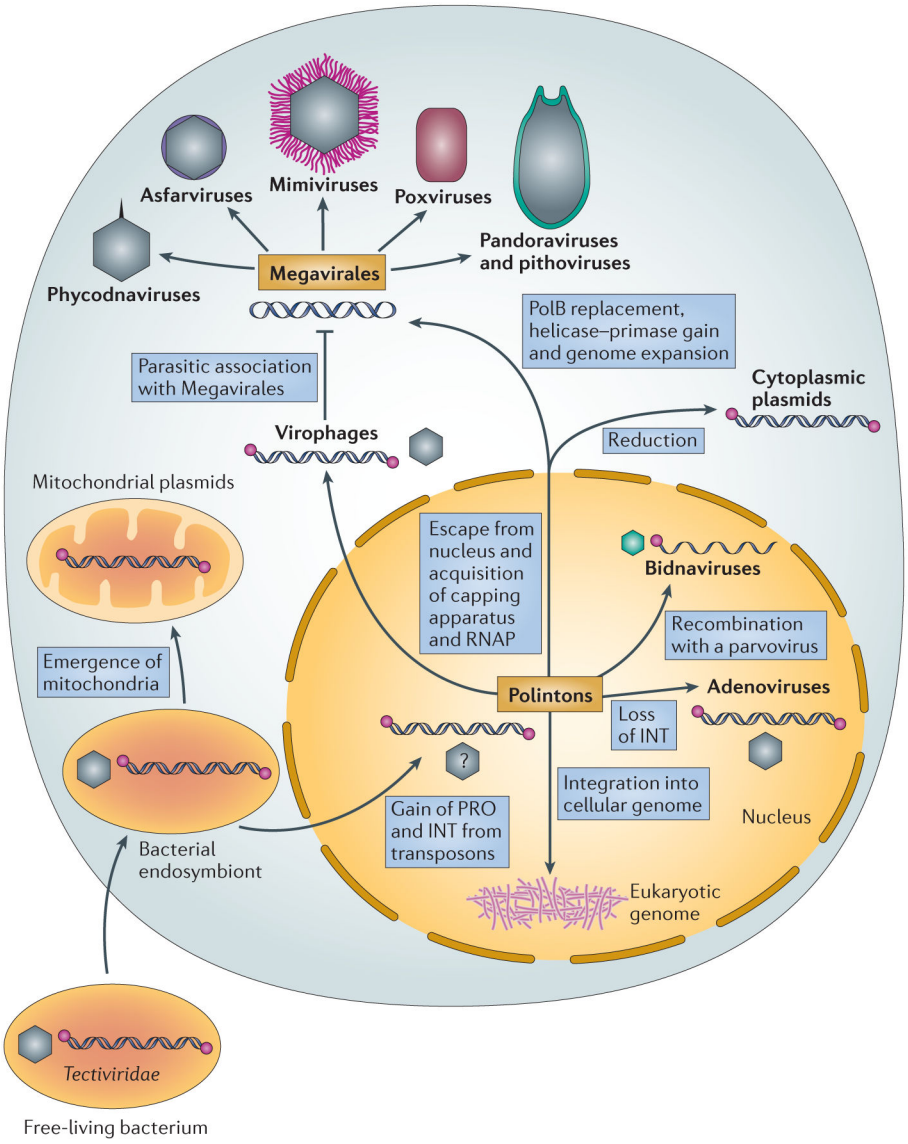

Figure 4. A hypothetical scenario for the evolution of various eukaryotic viruses and plasmids

In this scenario, the Polintons evolved from a tectivirus-like ancestor, which entered the proto-eukaryotic host within a bacterial endosymbiont that subsequently gave rise to the mitochondria. Polintons reside in the nucleus, and the key event in the emergence of both cytoplasmic plasmids and the proposed order ‘Megavirales’ was escape from the nucleus, which was associated with the acquisition of RNA polymerase (RNAP) and the capping apparatus from the host. The essential events in the evolution of Megavirales from the cytoplasmic Polinton-like ancestor were the replacement of protein-primed type B DNA polymerase (pPolB) with the RNA/DNA-primed PolB, the acquisition of the D5-like helicase–primase and genome expansion. Polintons are also implicated in the evolution of virophages, bidnaviruses and adenoviruses. The hexagons represent icosahedral capsids; in the case of Polintons, the capsids have been predicted to exist but have not yet been observed (indicated by a question mark). Double strands represent double-stranded DNA, whereas the

single-stranded DNA genome of bidnaviruses is shown as a single strand. INT, retroviral-like (RVE) family integrase; PRO, adenovirus-type cysteine protease.