

Mimivirus: the emerging paradox of quasi-autonomous viruses

Jean-Michel Claverie and Chantal Abergel

Structural and Genomic Information Laboratory, CNRS-UPR 2589, Aix-Marseille University, Mediterranean Institute of Microbiology, Parc Scientifique de Luminy, Case 934, 13288 Marseille Cedex 9, France

What is a virus? Are viruses alive? Should they be classified among microorganisms? One would expect these simple questions to have been settled a century after the discovery of the first viral disease. For years, modern virology successfully unravelled the huge diversity of viruses in terms of genetic material, replication mechanism, pathogenicity, host infection, and more recently particle structure, planet-wide distribution and ecological significance. Yet, little progress was made in understanding their evolutionary origin(s), as well as the fundamental nature of their relationship with the cellular world. Thanks to the recent studies on Mimivirus and other large DNA viruses, we are now entering a new era where the most basic concepts about viruses are revisited, including their true nature, how fundamentally different they are from cellular microorganisms, and how essential they might have been in the major innovations that punctuated the evolution of life.

Mimivirus: the dogma buster

Initially misinterpreted as *Bradfordcoccus*, a Gram-positive bacteria infecting *Acanthamoeba*, Mimivirus waited a decade for its true viral nature to be recognized [1]. The record-breaking size of the particle (750 nm in diameter) was the main emphasis of this very first report, as the historical characteristic feature used to distinguish viruses from other “microbes” was their ability to go through a filter with an average pore size of 500 nm in diameter. By becoming the first “non-filtering” virus, Mimivirus was breaking a century-old criterion. By comparison, the largest viral particles known at that time were those of Chlorella viruses or Poxviruses with dimensions of 200 – 350 nm. The anomalous size of Mimivirus particles probably precluded the discovery of its relatives in marine (or aquatic) environments, until recent metagenomic studies suggested that they might be quite abundant [2,3]. Indeed, virologists studying aquatic viruses had been traditionally isolating them by filtration through 0.3 μm pore-size filters, a protocol convenient for bacteriophages, but leaving Mimivirus, its relatives and many other undiscovered giant viruses, in the bacterial fraction. Breaking with a tradition more than a century old, Mimivirus also became the first virus with individual particles clearly visible under an ordinary light microscope.

The subsequent determination of the Mimivirus genome sequence resulted in the breaking of two additional dogma

strongly rooted in modern microbiology beliefs if less historical than the size barrier. First, with a dsDNA genome size of 1.2 Mb [4] predicted to encode more than 900 genes (recently increased to 981 [5]), Mimivirus was the first virus found to exhibit a larger genetic complexity than many intracellular parasitic bacteria. Second, Mimivirus was also the first virus to possess several of the most central components of the protein translation apparatus, including four amino-acyl tRNA synthetases that were later proven to be functional [6].

Other studies on Mimivirus led to the discovery of more unique, albeit less fundamental, features in its particle structure [7,8], genome delivery and packaging [9], and transcriptional regulation [5,10,11]. Having lost the straightforward criteria of size, genome simplicity, and translation competence as absolute boundaries separating cellular organisms from giant viruses, we are now facing the problem of redefining what we are prepared to call a “virus“. This new definition, or set of discriminating properties, will have to be flexible enough to allow the classification of the most unexpected microorganisms that will with no doubt turn up from the many ongoing and future microbiome explorations.

Is there a natural limit to the size and complexity of large dsDNA viruses?

With the discovery of Mimivirus, the historical (and operational) criterion of filterability (particle diameter <500 nm) has been broken, together with the notion that a virus genome has to be simpler than that of a cellular organism. A legitimate question is then: can we replace these traditional limits by new ones derived from biochemical and biological first principles? In other words, are there natural limits to a virus particle and/or genome size? Answering this question is philosophical, and central to guide our future search for more atypical viruses.

The general organisation of the capsids (or capsid heads) of dsDNA viruses (including phages infecting Eubacteria and Archaeobacteria) varies from highly symmetrical objects to a variety of irregular morphotypes (such as the round-shaped poxviruses [12], or the bottle-shaped archaeobacterial phages [13]). There is no apparent relationship between the size of the virus particle and its morphotype. Large capsids, for instance, might exhibit an icosahedral shape (i.e. Mimivirus, 500 nm in diameter) or an irregular one (i.e. Vaccinia virus, 350 nm in diameter). In addition, they might also differ by the presence of an outer lipid membrane (enveloped viruses), an internal lipid

Corresponding author: Claverie, J.-M. (Jean-Michel.Claverie@univmed.fr).

membrane (e.g. phycodnaviruses), or a peptidoglycan-like outer layer (Mimivirus). On the other side of the size spectrum, the particle (50 nm in diameter) of a recently described archaeal virus is a simple membrane vesicle enclosing an ssDNA genome, without detectable nucleocapsid-like proteins [14]. In our search for more atypical viruses, we must thus be prepared to encounter virions (nucleic acid-containing particles) hardly recognizable as such. Moreover, following the finding that Mimivirus particles are covered by a fibre layer of peptidoglycan [4,8,15] and given the capacity of viruses to exchange entire metabolic pathways with their hosts [16], it is not biologically absurd to predict that virions might be found encased in chitin (as found in the *Chlorella* cell-wall), calcite (as found in coccolithes), or silicate (as already proposed for virus-like particles associated with diatoms (M. Heldal and G. Bratbak, unpublished results).

Restricting the comparison to dsDNA viruses enclosed in a proteinaceous capsid exhibiting an icosahedral symmetry (i.e. similar to a regular solid made of 20 triangular faces), the known variation in size is truly amazing, ranging from 45 nm in diameter for a typical polyomavirus virus to 500 nm for the Mimivirus particle (not including the outer peptidoglycan layer). This factor of 11 in capsid diameter, converts to a factor of 1330 in terms of internal volume. The polyomavirus (e.g. SV40) genome is about 5 kb in size and encodes seven proteins [17]. Using the DNA packing density of the polyomaviridae as a reference, the Mimivirus capsid could easily incorporate a 6.5 Mb DNA molecule, encoding more than 6000 proteins! Thus, there is no physical limitation for giant viruses with capsid dimensions similar to that of Mimivirus to pack far more complex genomes. Even more so, as the DNA packing density in these viruses infecting eukaryotes is about ten times less than that found in bacteriophages, where it reaches 0.56 b per cubic nanometer [18]. At this density, the internal-most spherical compartment (about 400 nm in diameter) of the Mimivirus particle would allow 18 Mb of DNA to be packed, i.e. a genome larger than that found in many free-living unicellular eukaryotes (e.g. *Micromonas*, *Ostreococcus*, *Saccharomyces*), and comfortably larger than the reduced genomes of parasitic eukaryotes, such as 2.5 Mb in *Microsporidia* [19].

In summary, in the absence of a clear physical principle limiting the size of a viral genome that could be packaged in a sufficiently large capsid, the only natural limits one might think of are: (1) that a virus particle should remain smaller than the cell it infects (i.e. in the micrometre range); and (2) that a virus genome is expected to be smaller than that of its host. However, the example of the bacteriophage G (498 kb genome) infecting *Bacillus megaterium* (≈ 5.1 Mb genome) [20], indicates that the difference between the virus and its host might not be huge. We thus would not be utterly surprised if a giant virus with many more genes than Mimivirus and/or with a larger capsid was discovered in the near future.

Virus= virion: a common misconception

Since their discovery as filtering microbes, viruses have been defined by their virions, the viral particles produced

during infection [21]. The confusion between the virus and the virion is constant in the media (where the frightening pictures of prickly particles always illustrate articles on each new flu pandemic), but is also rampant in the scientific literature, such as when debating the position of viruses in the living world [22–24]. The crystallization of the tobacco mosaic virus (i.e. of its inert particles) contributed to the view that viruses should not be considered alive, despite their capacity to evolve and self-reproduce (two hallmarks of living entities) [21]. Indeed, viruses (as particles) do not exhibit any metabolic activity, a situation *a priori* incompatible with life. However, the detailed study of Mimivirus's replication cycle helped changing this viewpoint. The delivery of the Mimivirus's particle inner core within the host cytoplasm is followed by an eclipse phase after which a spectacular intracytoplasmic virion factory appears (Figure 1) [25–28]. As was already documented for poxviruses [29], these factories become the site of translation and transcription as well as replication of the viral genome [28], using the host's pool of metabolic precursors. At this stage, the functional resemblance between Mimivirus and an intracellular parasitic bacterium is striking, and the numerous cellular and biochemical functions involved in rebuilding from scratch this transient microorganism upon each infection cycle is what justifies the complexity of the Mimivirus genome. We thus proposed that the intracellular factory corresponds to the real virus, whereas the virion should be reappraised as the mere vehicle used to spread its genome from cell to cell [23,28,30]. Within this new conceptual framework, equating virus and virion is like confusing a seed with a tree, or an egg cell with a human being, even though both of them exhibit the same genome (Figure 2). This new way of looking at large DNA viruses infecting eukaryotes is making some progress in the community, where some authors extended it to viruses infecting Eubacteria or Archaeobacteria with the "virocell" concept, whereby the infected host cell becomes the true virus [24,31].

The notion that the Mimivirus virion factory should be considered a transient microorganism received additional support with the discovery that they could be "infected" by their own virus, Sputnik. At odds with previously described satellite viruses, Sputnik does not infect *Acanthamoeba* host cells in the absence of its helper Mimivirus, and the replication and transcription of its genome appear to be performed and regulated exclusively by the Mimivirus-encoded machinery [5,28]. Ending with the production of icosahedral particles, the whole Sputnik replication cycle takes place within Mimivirus intracytoplasmic factories [32–35], interfering with the production of Mimivirus particles. These properties led the authors to propose the (still controversial) concept of virophage to distinguish this *bona fide* "virus of virus" from satellite viruses co-infecting the same cellular host with a helper virus. This new kind of parasitism might be a common feature among large DNA viruses infecting eukaryotes, as another virophage (Mavirus) was recently discovered associated with CroV, a virus infecting *Cafeteria roenbergensis*, a single-cell flagellate from marine environments (C. Suttle and M. Fisher, unpublished results).

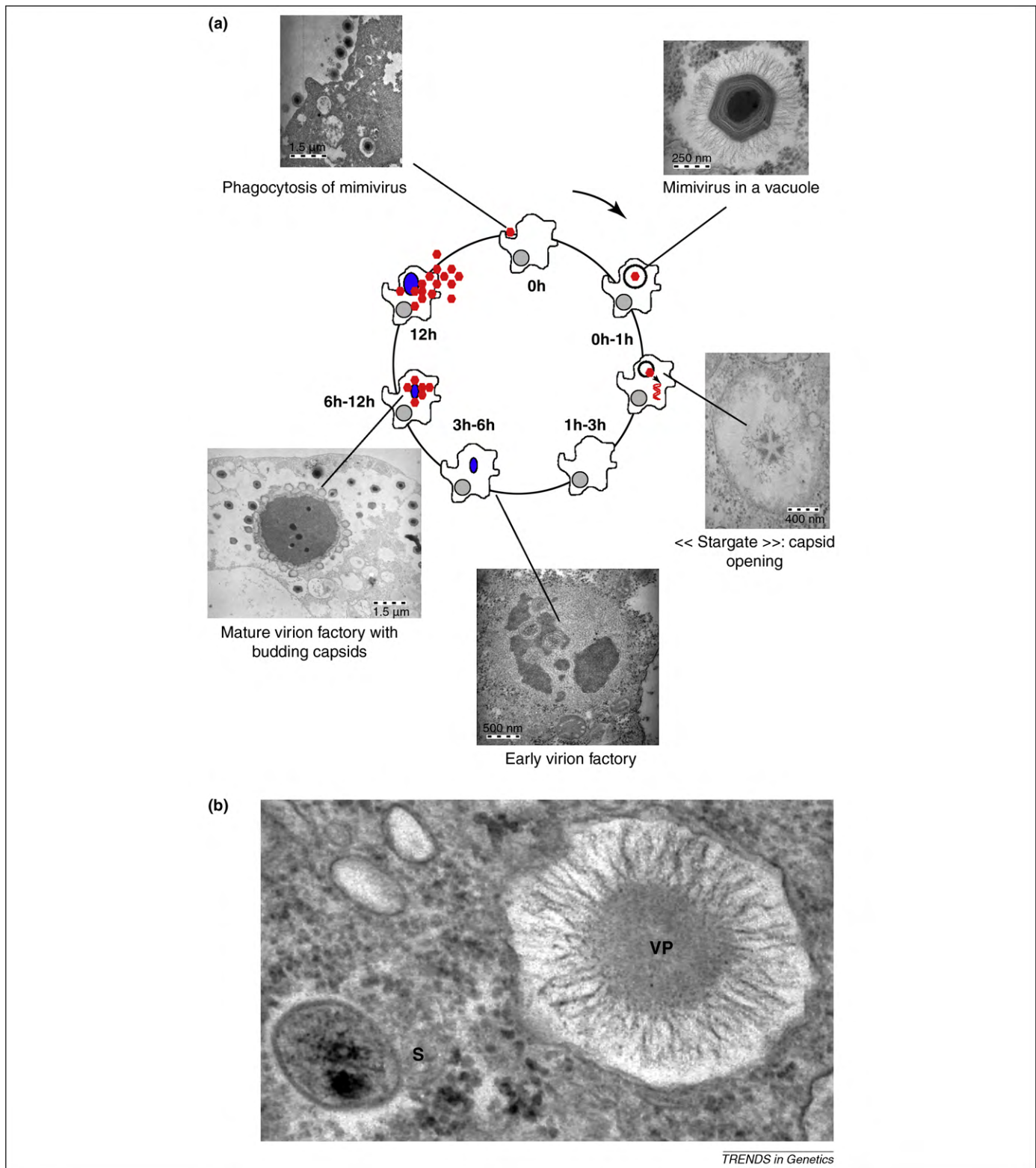


Figure 1. Life cycle of Mimivirus in *Acanthamoeba castellanii*. (a) Schematic representation (center) and transmission electron microscopy images illustrating the key steps of the infectious cycle. Mimivirus is represented as red hexagons, the virion factory as a blue oval, the cell nucleus as a grey circle, and the vacuole as a white circle. (b) Detail of an *Acanthamoeba* cell exhibiting two early phases of Mimivirus infection. One particle (VP, upper right) is still intact within a phagocytic vacuole while a second one (S, bottom left) is now in the cytoplasm, stripped of its outer layers to become the “seed” of the future virion factory.

Giant viruses (Giruses) versus parasitic cellular organisms: where is the frontier?

Once we accept the equality of the Mimivirus intracellular cytoplasmic virion factory with a transient microorganism, the paradox of the exceptional size of the Mimivirus

genome vanishes, as it bears no relationship to the simpler task of building a capsid, that we know can be encoded by a handful of genes [17]. A more meaningful comparison can now be made with the genome size and gene count of obligate intracellular parasitic bacteria such as *Chlamy-*

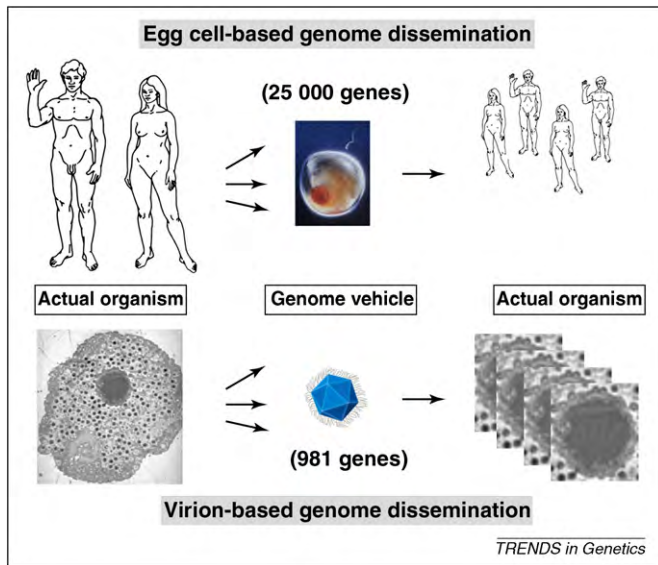


Figure 2. The true nature of large DNA viruses. The relationship between the virion factory (or the virocell [24]) to the virus particle, is the same as that between humans and their egg cells. Both propagate their cognate organism by packing their genome into a specialized “container” that initiates a developmental program leading to new organisms. The gene content is not correlated to the egg or virion stage, but to the complexity of the resulting organisms. We see the intracellular virion factories (the dark circle within the infected *Amoeba*, bottom left) as the true viral “microorganism”, which is the result of a developmental program initiated upon infection by a specialized genome dissemination device, the virion. Mimivirus was crucial in this change of perspective due both to the spectacular dimension of its virion factory (up to 6 μm in diameter) and its exceptional gene content (981 genes), an overkill for the simple task of encoding an icosahedral capsid. Both the egg and the virion are generic devices used to disseminate the genomes of vastly different organisms covering a large spectrum of phylogenetic distances. Consequently, their overall structure and morphology do not provide reliable clues to the evolutionary relationship of the cognate organisms.

dia, *Rickettsia*, or the lesser known *Trophyma*, all of them with genome size (~ 1 Mb) and gene count (< 1000) similar to those of Mimivirus [15]. As seen in all cellular parasites, these bacteria exhibit a clear tendency toward genomic reduction, with the loss of many biosynthetic capabilities that they compensate by obtaining the needed metabolites (such as amino acids, nucleotides or ATP) directly from the cytoplasm of the host cell. Interestingly, some of these (normally) obligate parasitic bacteria remain at the limit of the free-living life style, such as *Trophyma whipplei*, which is capable of growing in a specially designed axenic medium [36,37]. More extreme cases of genome reduction are exhibited by the vertically transmitted sap-eating insect symbionts such as the Gamma proteobacteria *Buchnera* (with genome sizes as small as 0.4 Mb)[38] and *Candidatus* (~ 0.16 Mb, coding for less than 200 proteins)[39]. In the latter case, the genome symbiont has lost all genes involved in cell envelope biogenesis and metabolism of nucleotides and lipids [39]. Is it still possible to draw a line between the virus and the cellular world given these bacterial cells displaying amazingly defective genomes and Giruses exhibiting an increasing diversity of biosynthetic pathways [4,16]? Both Giruses and (until now) bacterial symbionts encode their own DNA replication and transcription machineries. Giruses thus cannot be distinguished from minimal cells on the basis of the corresponding genes. The next possible frontier becomes the presence or the absence of a translation apparatus, thus dividing biological entities between

ribosome-encoding organisms (including eukaryotic, archaeal and bacterial organisms) and capsid-encoding organisms [40,41]. However, this criterion might not be as absolute as once thought [4,15]. On one hand, no basic principle precludes virus genomes from being packaged in a non-proteinaceous capsid, such as a simple lipid vesicle [14]. On the other hand, the dogma that viruses entirely delegate the translation of their proteins to their host is already broken. We found nine genes central to the protein translation process in the Mimivirus genome: four aminoacyl-tRNA synthetases, four translation factors and one peptide chain release factor. All these genes are expressed during the infectious cycle [5], and the function of two of them has been directly validated [6]. tRNAs are also found in many large DNA viruses, such as in Mimivirus, where their expression has been validated [11]. As for the genes encoding the ribosomes themselves, it is true that they remain, for now, the privilege of cellular organisms. Even the most reduced bacterial symbionts appear to have retained all the genes necessary to build their own ribosomes (ribosomal proteins, 16S, 23S and 5S ribosomal RNAs, and tRNA genes for all 20 amino acids) [39]. However, this requirement might not be absolute in the world of parasitic eukaryotes, within which mechanisms for the import of ribosomal proteins seem to exist [42]. A complete set of ribosomal genes might thus not be required in the most reduced parasitic or symbiotic cellular organisms. By contrast, nothing would preclude a giant virus from encoding some of its own ribosomal components; for instance, to more selectively translate its own mRNAs. This could actually be the role of the two tRNA-modifying enzymes and of the mRNA CAP-binding protein encoded by Mimivirus. For bootstrapping their infectious cycle independently of the host cell nucleus (Figure 1b), Poxvirus (or Mimivirus) particles incorporate multiple copies of ready-to-go transcription complexes [5,43]. For the same purpose, a Mimivirus particle could easily fit a few ribosomes (as already documented for Arenaviruses packaging host ribosomes) that measure only 30 nm at their largest dimensions. Thus, there is no basic principle that would preclude virion factories from using ribosomes encoded (at least partially) by a Girus genome. The apparent incompatibility between the presence of encoded ribosomes and the world of viruses is thus awaiting an explanation. This puzzling dichotomy, if it remains absolute in the future despite the discovery of more giant viruses, should be taken into account in any scenario we might propose for their evolutionary origin.

In summary, the convergence between some cellular organisms increasingly host-dependent and harbouring less than a minimal genome [39] and quasi-autonomous Girus encoding increasingly cell-like virion factories and simply using the cytoplasm as a rich medium, suggests that the sole analysis of a gene content might not be sufficient, in the most extreme cases, to distinguish between them. In terms of genomic complexity, a continuum appears to exist between the world of viruses and parasites or symbionts derived from cellular organisms.

The evolutionary hypotheses on the origin of Giruses

Despite its many unique features [28], Mimivirus (and its close relative [32,44]) appears to belong to a class of large

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dsDNA viruses referred to collectively as nucleo-cytoplasmic large DNA viruses (NCLDV) [45] and infecting a variety of eukaryotic hosts. NCLDVs include Poxviruses, Iridoviruses and Phycodnaviruses as major representatives. A maximum-likelihood reconstruction of NCLDV evolution yielded a set of 47 conserved genes [45]. The existence of these “core” genes suggests strongly that they were present in the genome of their last common ancestor, a virus encoding a complex machinery of replication, transcription, morphogenesis, and metabolic pathways making it relatively independent from host cell functions [45]. However, the existence of vastly different genome sizes (from 1.2 Mb for Mimivirus down to 102 kb for the smallest Iridovirus) among NCLDVs, the erratic distribution of these core genes, and the lack of a bona fide “core genome” as initially defined [46] (with only five genes conserved in all analyzed genomes) suggests a scenario more complex than vertical descent from a common ancestor coupled with lineage-specific gene losses [45]. In contrast, vastly different viruses infecting hosts from different domains (Eubacteria, Archaea and Eukarya) share homologous capsid proteins and/or ATPases for genome packaging, suggesting that they evolved from a common ancestral virus, eventually predating the last universal common ancestor [47]. In the wake of previous hypotheses linking DNA viruses to the emergence of the eukaryotic nucleus and the shift from RNA to DNA cellular genomes [48–51], we propose an alternative hypothesis combining a vertical descent scenario with the trapping of an ancestral DNA virus and the possibility for proto-nuclei to revert to an infectious viral-like state, as still observed today in some parasitic red algae (Figure 3) [52]. This new scenario accounts for the wide variation in genome size and gene content among NCLDVs, together with the sharing of homologous capsid components by NCLDVs and many other unrelated viruses. In this framework, the viral particle (i.e.

the capsid protein and the packaging ATPase), although the most characteristic feature of the virus world, becomes a mere vehicle successfully selected to package parasitic genomes with vastly different evolutionary histories, providing a natural explanation for the lack of correlation between particle structure and viral genome complexity (Figure 2).

Therefore, the highly complex Mimiviridae would have originated late, near the completion of this iterative evolutionary process, packaging a large complement of ancestral genes (eventually predating the Cambrian explosion leading to modern organisms [53]). Reductive evolution, a well documented process common to all intracellular parasites [54,55], would then be responsible for the progressive streamlining of the genome of the ancestral Mimiviridae to its present state [4,28].

Some authors remain strongly opposed to the hypothesis of any highly complex ancestor virus [24], and insist on explaining the anomalous size of Mimivirus genome by an exceptional propensity to acquire genes from its host and other viruses by lateral transfer [22,56,57]. This conservative model suffers three main shortcomings: (1) the lack of similarity of most Mimivirus genes (> 60%) with extant cellular (or viral) genes [4,28]; (2) the small proportion (~10%) of Mimivirus genes due to recent horizontal transfer [58]; and (3) the violation of the rule that intracellular parasites are invariably submitted to genome reduction as observed in the many *Acanthamoeba*-infecting bacteria that have been studied [59]. In this context, the oversized capsid used by Mimivirus to pack its genome is consistent with the notion that it was inherited from a Mimiviridae ancestor possessing a much larger genome.

Concluding remarks

Since the initial discovery of Mimivirus, many phylogenetic analyses have been published to support or invalidate

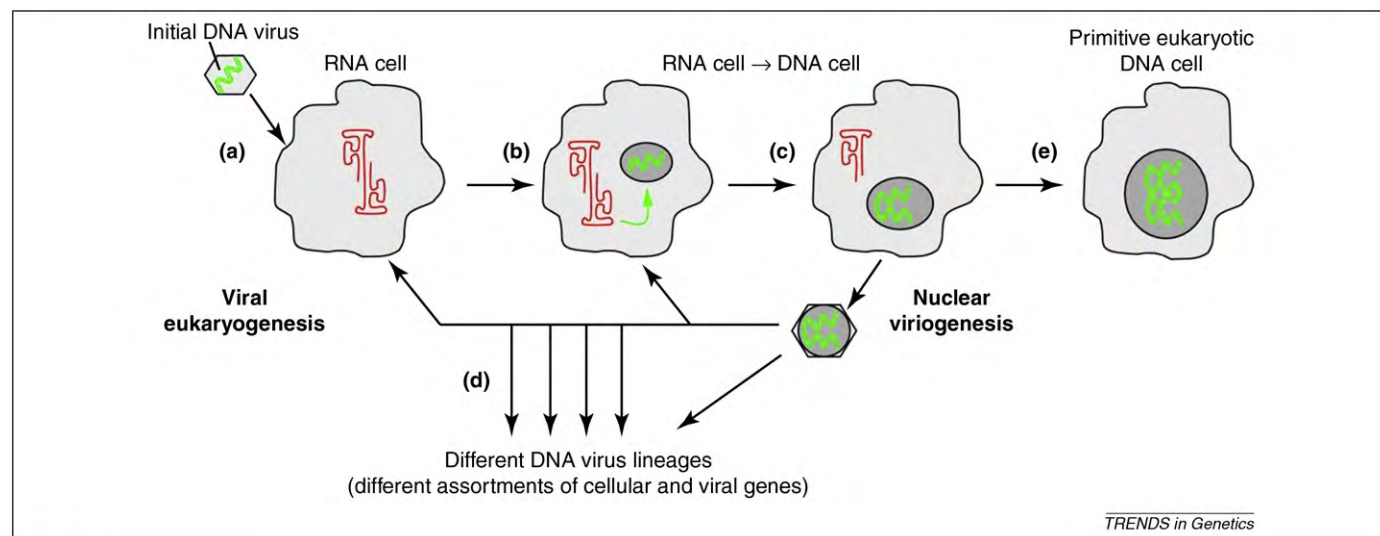


Figure 3. An iterative model for the origin of the eukaryote nucleus and the simultaneous emergence of various NCLDV families. (a) A primitive DNA virus (a bacteriophage ancestor) gets trapped within an RNA cell initiating a proto-nucleus. (b) Cellular genes are progressively recruited to the proto-nucleus, pushed by the selective advantages of DNA genomes. (c) This situation remains unstable for a while with some of the proto-nuclei reverting to a viral state (generalizing the use of the original capsid structure as a genome vehicle). (d) These viruses infected other cells at various stages of their rapid ongoing evolution. (e) The emergence of nucleated cells with biochemically more stable DNA genomes might have coincided with the end of the pre-darwinian era [60] where a collective mode of evolution (characterized by unstable proto-cellular organisms overwhelmed by too frequent horizontal gene transfers) switched to a cellular mode of evolution (with the dominance of gene inheritance by vertical descent, both in the cellular and viral world). This hypothetical scheme provides a mechanism for the emergence of various overlapping virus lineages, all using the bacteriophage inherited capsid structure, predating the emergence of the eukaryotes and exhibiting various reassortments of viral and ancestral cellular genes. (The figure is adapted from Ref. [30].)

its putative position in the Tree of Life. We now believe that studies based solely on sequence comparison cannot put an end to this ongoing controversy. To understand the origin of Mimivirus as well as its eventual link with the emergence of the cell nucleus, we must now decipher the developmental-like program that transforms its metabolically inert particle to a full-blown virion factory. Such functional studies could lead to invaluable insights into the origin of all life forms.

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