Giant mimiviruses escape many canonical criteria of the virus definition

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ABSTRACT

Background. The discovery of mimivirus in 2003 prompted the quest for other giant viruses of amoebae. Mimiviruses and their relatives were found to differ considerably from other viruses. Their study led to major advances in virology and evolutionary biology. ***Aims.*** We summarized the widening gap between mimiviruses and other viruses. ***Sources.*** We collected data from articles retrieved from PubMed using as keywords "giant virus", "mimivirus", and "virophage", as well as quoted references from these articles. ***Content.*** Data accumulated during the last 15 years on mimiviruses and other giant viruses highlight that there is a quantum leap between these infectious agents which complexity is similar to that of intracellular microorganisms and classical viruses. Notably, in addition to their giant structures and genomes, giant viruses have abundant gene repertoires with genes unique in the virosphere, including a tremendous set of translation components. The viruses contain hundreds of proteins and many transcripts. They share a core of very central and ancient proteins but their genome sequences display a substantial level of mosaicism. Finally, mimiviruses have a specific mobilome, including virophages that can integrate into their genomes, and against which they can defend themselves through integration of short fragments of these invaders' DNA. ***Implications.*** Mimiviruses and subsequently discovered giant viruses have changed the virus paradigm and contradict many virus definition criteria delineated for classical viruses. The major cellular hallmark that still lacks in giant viruses is the ribosome, including both ribosomal protein and RNA encoding genes, which makes them *bona fide* microbes without ribosomes.

TEXT

INTRODUCTION

Giant viruses of amoebae were discovered in 2003 (Table 1) [1]. The characterization of their first representative, Acanthamoeba polyphaga mimivirus (APMV), prompted the quest for other similar infectious agents. During the last 15 years, nine new groups of giant viruses have been described [2]. They include marseilleviruses, pandoraviruses, pithoviruses, faustoviruses, molliviruses, kaumoebaviruses, cedratviruses, pacmanviruses and orpheoviruses [2,3]. Giant virus names have been chosen by their discoverers for various reasons, due to specific features including morphological (as for the case of mimivirus, an acronym for Mimicking Microbe Virus in memory of "Mimi the amoeba", example of the story of the evolution told by his father to one of us (DR)), or to the geographical location of virus isolation or discovery (as for the case of Marseillevirus isolated in Marseille, France) [4]. Their particles have a size ranging between 200 and 2,300 nm and their genomes harbor 444-2,544 genes [2,5]. Thus, giant viruses are as big or even bigger than some bacteria and they encode similar or greater number of genes. Beyond, data accumulated highlight that there is a quantum leap between these infectious agents and classical viruses, and some contain an almost comprehensive translation apparatus, although they lack ribosomes. In addition, the first viruses of virus to be discovered, named virophages, were found to replicate in mimivirus factories and their DNA can integrate into the genomes of mimiviruses, in addition to mimivirus-specific transposons named transpovirons. Mimiviruses and virophages strongly interact between each other, through sequence transfer, integration of virophages DNA into giant virus DNA, and defense mechanisms of mimiviruses against virophages. Therefore, giant viruses, virophages and their relationships represent new challenges in the field of virology. We focused here on the widening gap between mimiviruses and other

viruses.

MIMIVIRUSES

APMV, the prototype virus of family *Mimiviridae*, was considered for years to be a Gram positive coccus because such large viruses were unknown at that time. Then, no PCR products were obtained using universal primers targeting bacterial 16S ribosomal DNA, but electron microscopy finally revealed ≈500 nm-large icosahedral viruses with ≈120 nm-long fibers. This first work inaugurated the world of *bona fide* "microbes" without ribosomal DNA [6]. Since then, the family *Mimiviridae* has considerably expanded through the culture of mostly environmental water samples on *Acanthamoeba* spp. [2,7]. These mimiviruses have capsids with a diameter ranging between 370-600 nm and their genomes are 1.02–1.52 megabase pairs (Mbp) in length and encode 930–1,425 putative proteins [2]. The main phenotypic and genotypic features of mimiviruses are described in Box 1. Three groups A, B and C of mimiviruses replicating in *Acanthamoeba* species were delineated based on phylogenomic analyses [8]. These giant viruses are common in water and soil worldwide, and were in a few cases detected and even isolated from animals and humans [2,9,10].

Since 2010, distant relatives of mimiviruses of *Acanthamoeba* have been described, which infect common protists, flagellates and algae, mostly in marine waters [11]. They include Cafeteria roenbergensis virus (CroV) [12], the first one to be discovered, as well as Phaeocystis globosa virus and others [11,13-19]. Such mimivirus relatives are suspected to be paramount components of our biosphere [20,21]. Recently, the 0.9-1.6 Mpb genomes of four putative giant viruses, named klosneuviruses and related to amoeba mimiviruses, have been assembled from environmental metagenomes [22]. Subsequently, a new giant virus (Bodo saltans virus) infecting *Bodo saltans*, an aquatic protozoan, was isolated and classified as a klosneuvirus [23]. Two mimivirus-like strains were also isolated within or using *Saccamoeba lacustris* strains, from the bark of plane trees [24].

In 2018, two new mimiviruses, tupanvirus soda lake and tupanvirus deep ocean, were described [5]. They were isolated in Brazil from a "soda" lake and oceanic sediments, respectively. These viruses infect *Acanthamoeba* strains, but also *Vermamoeba vermiformis* and a wide range of protists *in vitro*. The viruses have icosahedral capsids similar to those of other mimiviruses of amoebae and a tail also covered with fibers that is the longest amongst viruses, since capsid and tail can reach together 2.3 μm in length. The genomes of these two tupanviruses harbor 1.44 and 1.52 Mpb and encode 1,276 and 1,425 genes, respectively.

VIROPHAGES AND THE MIMIVIRUS MOBILOME

The isolation of a second mimivirus in 2008 led to the discovery and description of a small (≈50 nm-large) icosahedral virus (strain Sputnik), the first virus of virus [25]. It was named a virophage by analogy with bacteriophages. Indeed, it was unable to multiply in the absence of mimivirus, replicated in the mimivirus factories, slowed down the mimivirus replication and was commonly associated with production of morphologically abnormal viruses. Sputnik particles were found associated with mimivirus fibers [25-27]. Both virophages and mimiviruses are produced within same viral factories. Sputnik appears to hijack the mimivirus factory, being detected inside it and often produced before the giant virus or at a distinct location. The 18,343 bp long circular dsDNA genome of Sputnik contains 21 genes including 14 ORFans and 7 ORFs with various putative origins including eukaryotic, bacterial, viral or plasmidic (Figure 1). Such chimeric gene repertoire and the presence of putative integrases and transposases led to hypothesize that this virophage might represent a vehicle of genes. Another virophage, Mavirus (for Maverick virus) was isolated in association with CroV [28]. It also reduces amoebal cell lysis and production of its associated giant virus. Mavirus has a 19,063 bp circular dsDNA genome that encodes 20 proteins. Similar genome length and structure and a close evolutionary relationship were noticed between mavirus and the self-

synthesizing maverick or polinton transposable elements [28]. The integration of provirophages in the genome of *Bigelowiella* isolates, an alga, was also described, suggesting that these cells may be infected with mimiviruses [29]. Overall, virophages have been isolated or detected by metagenomics in samples, mainly water, collected worldwide [30-32]. They have 35-74-nm-large icosahedral capsids, and genomes encoding 16-34 genes. They were recently classified in a new viral family, named *Lavidaviridae* [33].

The mobilome represents all the kinds of mobile genetic elements, which are key players of genetic evolution: it comprises transposable elements, plasmids, introns and inteins, as well as viruses, [34]. The mimivirus specific mobilome corresponds to provirophages, transpovirons, inteins and introns. Whereas classical viruses are considered as a mobilome component, giant viruses have their own mobilome [34], another feature that they share with cellular organisms [35] (Figure 2). Virophages are part of the mimivirus mobilome. They can integrate into mimivirus genomes as pro-virophages [34]. This is also the case for transpovirons, a new type of transposons specific to mimiviruses. They are composed of linear DNAs of about 7 kbp containing 6-7 genes, two of which are shared with virophages [34]. They depend on mimiviruses for their replication, being concentrated around the mimivirus factories during the replicative cycle, and they accumulate in the amoebal cytoplasm and the mimivirus and virophages virions.

MIMIVIRUS SYMPATRIC LIFESTYLE IN AMOEBAE THAT ARE SITES OF GENERALIZED SEQUENCE TRANSFERS

The tremendous size and composition of giant viruses that infect protists have been linked to their sympatric lifestyle, consisting in their survival and replication in a microbial community [44]. The genomes of giant viruses are mosaics containing genes of eukaryotic, bacterial, and/or archaeal origin (Figure 1) and are considerably reshaped by lateral sequence transfers,

149 in addition to gene duplication [42,45-48]. This is exemplified by mimivirus (Figure 1), and
150 by tupanvirus whose genes were inferred to have multiple origins, therefore evolutionary
151 histories, warranting the representation of tupanvirus evolution as a rhizome rather than as a
152 tree [5,49]. *Acanthamoeba* spp. and other free-living amoebae and phagocytic protists are
153 cosmopolitan and predominant in the biosphere [50-53]. They can harbor a variety of
154 intracellular amoeba-resisting microorganisms [52]. As an illustration, an *Acanthamoeba* sp.
155 was isolated from a contact lens rinsing liquid together with a deltaproteobacterium
156 (*Candidatus* Babela massiliensis), an alphaproteobacterium bacillus, a mimivirus and a
157 virophage [54]. *Cafeteria roenbergensis*, the CroV host, also feeds on bacteria and viruses,
158 and its genome was shown to harbor a 38-kbp fragment with 14 ORFs most similar to
159 bacterial genes involved in carbohydrate metabolism [12,55]. In addition, it was observed that
160 obligate intra-amoebal parasites exhibit larger genomes than their intracellular relatives [43].
161 Furthermore, subculturing the APMV prototype isolate 150 times in a germ-free amoebal host
162 (in allopatry) was associated with a dramatic genome reduction (by ≈16%) [27].
163 In phagocytic protists, viruses and bacteria have to struggle for life, and they need a
164 substantial and adapting gene armory to outcompete with, or at least resist to other
165 microorganisms and genetic elements [44]. *Acanthamoeba* spp. may therefore be viewed as
166 an ”adaptative zone” for giant viruses and virophages. The fight between microorganisms
167 living in sympatry inside *Acanthamoeba* spp. and the host itself follows an evolutionary law
168 described in 1973 by L. Van Valen [56]. To illustrate this theory, he borrowed a scene from
169 Lewis Caroll’s book *Through the looking-glass* where Alice runs as fast as she can with the
170 Red Queen through a chessboard, but nonetheless surprinsingly remains in the same place.
171 According to this "Red Queen hypothesis", co-existing organisms play a zero-sum game,
172 where useful or necessary weapons and shields can vary from time to time, there is no single
173 or chief competitor, and no victory is definitive as enhanced or new adversaries continue to

emerge. In the setting of continuous sequence transfers promoted by contacts between genetic material from the intra-amoebal microorganisms during their multiplication or replication, each microorganism may act as a tinkerer [57] by using sequence gains and losses to conceive new attack or defense tools. Moreover, new adaptations may occur at the expense of previous changes. This paradigm parallels that of the representation of genetic evolution as a rhizome, which is devoid of a center and can grow through gaining or loosing one or several elements [49,58]. It can be illustrated by several recently described defense mechanisms involving mimiviruses, including the defense of mimiviruses of amoebae against virophages.

MIMIVIRUS AND VIROPHAGE DEFENSE MECHANISMS

Following the observation that mimiviruses of group A, but not B-C, were resistant to the Zamilon virophage [59], the means by which mimiviruses A could defend against this virophage were investigated. This investigation was conducted by analogy with the defense strategy of bacteria and archaea against viruses through CRISPR-Cas systems, which consists in the integration into their genomes of fragments of these invaders' DNA ("cannibalism") [60]. Thus, short specific Zamilon DNA fragments were searched for in the genomes of mimiviruses A. Four copies of a 15 nucleotide-long Zamilon DNA fragment, named MIMIVIRE for "mimivirus virophage resistance element", were detected within an operon (Figure 1). This sequence was absent from the genomes of Zamilon-sensitive mimiviruses B-C. The mechanism of this resistance might rely on nucleic acid sequence recognition and confer immunity against virophages. The MIMIVIRE operon includes one gene that contains the 15 nucleotide-long Zamilon sequences and two genes annotated as a helicase and a nuclease, whose functions were experimentally validated. They may allow the cleavage of a foreign nucleic acid. The restoration of mimiviruses A sensitivity to Zamilon virophage was observed by independently inhibiting these three genes by RNA interference. Although it has

been primarily underlined that the MIMIVIRE genes may not be related to a CRISPR-Cas system [61], it was shown later that it contained at least one Cas4-like protein based on structural and mechanistic analyses [62]. The integration of sequences from invaders as a defense mechanism appears to be a general phenomenon in biology. Interestingly, the presence of ribonucleases (RNases) H was recently detected in mimivirus genomes [63]. RNases H and RNase H-like enzymes are involved in most or all currently known defense mechanisms against transposable elements, viruses, and extracellular pathogens.

Mavirus integrated as a pro-virophage into the *Cafeteria roenbergensis* genome was recently shown experimentally, subsequently to host superinfection by CroV, to be transcribed, then to replicate without being directly detrimental to CroV replication [64]. Nevertheless, CroV replication suppression and enhanced survival of the host population were thereafter observed, suggesting the acquisition of a protection conferred by Mavirus reactivation. Other putative defense mechanisms of mimiviruses against sympatric organisms were recently suggested for *Bodo saltans* virus [23]. Competitive interference between different *Bodo saltans* virus strains or related viruses co-infecting a same host cell may occur through the site-specific homing endonucleases encoded by introns and inteins that have invaded essential genes of this mimivirus, including DNA-dependent RNA polymerase subunits. Indeed, these endonucleases can cleave the locus that is unoccupied in viral genomes lacking the intron or intein.

THE GROWING MISMATCH BETWEEN GIANT VIRUSES AND THE CRITERIA OF DEFINITION OF CLASSICAL VIRUSES

Mimivirus initially resisted identification for more than a decade because its phenotype did not match with that of viruses. It was considered as a microorganism (a Gram positive coccus), i.e. an organism visible only using optical microscopy [65]. Similarly,

pandoraviruses were considered as microorganisms and named *Acanthamoeba* endosymbionts due to their size (Figure 3) [66]. Later, its was shown that giant viruses of amoebae had hallmark features of cellular microorganisms and, *de facto*, most criteria of definition of viruses did not apply to them [35].

The virus concept grew on the initial observations by Ivanowsky and Beijerinck that infectious agents smaller than microorganisms, not ultrafilterable themselves, may exist and transmit infections [67,68]. Congruently, these infectious agents were not visible by light microscopy that allows seeing shapes larger than 200 µm. This paradigm was applied to viruses until the APMV discovery (Table 2). The modern concept of virus was delineated in 1957 by Lwoff [69], although intracellular bacteria and viruses were grouped together in microbiology books until the 1970s. Lwoff stated that viruses contain only one type of nucleic acid, DNA or RNA; reproduce only from their genetic material, being unable to grow and divide; and do not harbor in their genomes information encoding an energy production system. The viral capsid was described a few years later [70]. Messenger RNAs are in fact detected in giant viruses, many having been detected in APMV particles [41]. Therefore, these viruses contain both DNA and RNA. While giant viruses do not multiply through binary fission, those with ovoid viruses (including pandoraviruses, pithoviruses and cedratviruses) have a weird assembly stage during which the viruses tegument seems to grow progressively while enclosing gradually its content [37]. Moreover, giant viral factories look like morula from *Chlamydia* spp. [71]. The emblematic lack of enzymes involved in intermediate metabolism was recently challenged by the finding in a green alga-infecting mimivirus named Tetraselmis virus 1 of two key fermentation genes encoding a pyruvate formate-lyase and a pyruvate formate-lyase activating enzyme [17]. Besides, some obligatory intracellular parasites among which the small bacteria *Carsonella ruddii* are devoid of a complete ATP generation machinery [72]. Regarding the capsid, mimiviruses, as a majority of giant viruses,

harbor one, which is pseudo-icosahedral [1]. Nevertheless, pandoraviruses are devoid of recognizable capsid gene [37], while pithoviruses harbor a capsid gene but no capsid protein detected by proteomics. They are also devoid of any known capsid morphology, as are cedratviruses and orpheovirus [3,73,74].

Furthermore, emblematic elements that are lacking among classical viruses are protein synthesis components. Ten translation-related genes were described in the APMV genome [41]. They corresponded to aminoacyl-transfer RNA (tRNA) synthetases or translation factors. In addition, six tRNA-encoding genes were present. It was later shown that RNA silencing of the gene encoding a putative translation initiation factor (R458) was associated with a prolonged eclipse phase and a delay in the appearance of the mimivirus factory, along with modulation of expression of 32 mimivirus genes [75]. Recently, the mimivirus translation component set showed a dramatic expansion with the assembly of genomes of putative non-isolated mimivirus relatives, named klosneuviruses [22], then with the *Bodo saltans* virus discovery [23]. Nevertheless, current record holders are tupanviruses whose genomes harbor the most complete set of translation-related genes of the virosphere, with all 20 aminoacyl-tRNA synthetases, and 67-70 tRNAs associated with 46-47 codons [5]. In addition, there are two different copies of an intronic region of 18S ribosomal RNA in the tupanvirus genome, and such sequences were shown to be present in other mimiviruses of amoebae. When excluding ribosomes, tupanvirus genomes harbor as many translation-related genes as *Carsonella ruddii*, a bacterium, *Nanoarchaeum equitans*, an archaeon, and, in the case of Tupanvirus deep ocean, *Encephalitozoon cuniculi*, an eukaryote. Thus, the major cellular hallmark that still lacks in giant viruses is the ribosome, including both ribosomal proteins and RNA-encoding genes. Overall, there is hence a quantum leap between classical viruses and mimiviruses and other giant viruses that have a complexity similar to that of intracellular microorganisms.

THE PRESENCE AND SIGNIFICANCE OF GIANT VIRUSES IN HUMANS

Beyond being currently considered as common in water and soils worldwide, giant viruses have been detected in human samples in several studies and by several teams. Notably, hints of their presence in humans have been increasingly reported through metagenomic analyses [2,76-79]. This involved sequences related to mimiviruses and virophages but also to marseilleviruses and more recently discovered giant viruses. Various other techniques have contributed to identify the presence of giant viruses in humans, including serologies, immunohistochemistry and immunofluorescence techniques, PCR and fluorescence *in situ* hybridization (FISH) [2,80-84]. A critical step in studying the presence and significance of giant viruses in humans was their isolation from human samples. A marseillevirus was in 2012 the first giant virus of amoebae to be isolated from a human, in the feces from a young healthy Senegalese man [85]. Then, a mimivirus was isolated from the bronchoalveolar fluid and the feces of two pneumonia patients [82,86], and from the urine of a kidney-transplant recipient in whom no role of the giant virus could be speculated in the patient's symptoms that consisted in acute diarrhoea and dyspnoea [9]. Based on serological assays, molecular tests and culture isolation, mimiviruses were suspected to be linked to pneumonia [82], whereas marseilleviruses were mostly detected in the blood and the lymphoid tissues and associated with adenitis and Hodgkin lymphoma [83,84] . Besides, mice inoculated intracardially with APMV developed histopathologically proven lesions of pneumonia [87] and a 30-day long persistence of Marseillevirus but no disease was observed in rats and mice inoculated parenterally, intraperitoneally or by aerosolization [88]. Overall, although the presence and clinical significance of giant viruses in humans are still controversial issues [89,90], they represent an emerging field that deserves broader analyses and increased consideration in future studies.

CONCLUSION

Mimiviruses and subsequently discovered giant viruses have changed the virus paradigm and contradict many virus definition criteria delineated for classical viruses. An opposite change recently occurred when ultrasmall bacteria were discovered, and found to lack genes involved in metabolic processes formerly considered as essential [91,92]. Mimiviruses can be currently considered as microorganisms lacking a canonical ribosome. Nevetheless, it is possible that an alternative translation device may have existed, discarded later on during the evolutionary process. Furthermore, future studies are warranted to gain a better knowledge on the presence and significance of giant viruses in humans.

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Conflicts of interest

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BOXES

Box 1. Main phenotypic and genotypic features of mimiviruses

The **capsid** of mimiviruses of amoebae is an almost perfect icosahedron, except at one of its vertices**,** which is a gateway for the liberation of the genome and is covered with a five-pointed star-shaped structure [1,93]. This capsid comprises proteins having a structure of the type "double jelly roll fold".

The viruses can carry on its surface highly glycosylated protein **fibers**, the constitution of which is not yet completely elucidated, that seem to intervene in viral entry into amoebae, and have a capacity of attachment to other organisms [94]. Under the capsid, surrounded by an internal lipid membrane and fibers, there is a spherical compartment bounded by a double lipid layer and containing the genome and proteins [1,93].

Mimivirus **genomes** are linear double-stranded DNAs that are very rich in A+T (≈72% of the bases for the Mimivirus prototype isolate) with a protein coding density of ≈90% [41].

The **gene repertoire** of mimiviruses contains so-called **"core" genes** [41], which have homologs in poxviruses, asfarviruses, phycodnaviruses, ascoviruses and iridoviruses joined together in 2001 in the group of large nucleocytoplasmic DNA viruses [95]. Among the approximately 200 identified "core" genes, there are some that show the monophyly of all these viruses. Fifty core genes conserved in all or a part of these viruses have been assigned to the genome of a common ancestor [95,96]. Phylogenetic and phyletic analyses of the mimivirus gene repertoires, particularly of genes that have homologs in cellular organisms, and of the protein folds detected in these viruses, support an early origin of the mimiviruses and other giant viruses [97-99].

Moreover, mimivirus genomes harbor significant proportions of **duplicated genes**, of so-called "**ORFans**" (genes with no known homolog in sequence libraries) accounting for about 48% of all genes, and of genes that are predicted to have been involved in **lateral exchanges of sequences** with other organisms, including their amoebal host (Figure 1) [41]. Promoters, introns and inteins have also been predicted.

Functions not predicted before in viruses were revealed in mimiviruses. Emblematically, some mimivirus genes encode aminoacyl-RNA transfer (tRNA) synthetases, factors involved in translation, and tRNAs. Other unique genes amongst viruses are involved in nucleotide synthesis, amino acid metabolism, post-translational modifications, lipid and carbohydrate metabolism, or DNA repair.

There are genes whose presence suggests a **high degree of autonomy for replication,** with respect to their host cell. These genes encode, in particular, DNA polymerases, helicases and primases associated with DNA replication, RNA polymerase subunits, transcription factors, Holliday junction resolvers and topoisomerases involved in the processing and maturation of DNA, ATPase pumps for packaging DNA, chaperone molecules involved in capsid assembly, and capsid proteins [96].

Polyadenylated messenger RNAs have been detected, often with stem-loop structures towards their terminal end [100].

FIGURE LEGENDS

Figure 1. Schematics of the mosaicism of the Mimivirus and Zamilon virophage genomes, and of the MIMIVIRE defence system

Colours integrated in the circular gene data visualizations indicate if the most similar sequences for Mimivirus and Zamilon genes in the NCBI GenBank non-redundant protein sequence database belong to an eukaryota (blue), a bacterium (green), an archaeon (purple), a virus (red), or if no significant hit was found (yellow). Similarity searches were performed with the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For Mimivirus and Zamilon genes, BLAST hits corresponding to members of family *Mimiviridae* or *Lavidaviridae*, respectively, were excluded. The figures were generated using an in-house bioinformatic pipeline. Viral genes were distributed according to their position in the genomes either on a whole circle (a) or on a semicircle with their rearrangement on the remaining part of the circle according to the classification of their most similar sequence as eukaryotic, bacterial, archeal, or viral, or the absence of significant BLAST hit (b).

Figure 2. Mobile genetic elements comprising the mimivirus mobilome

Figure 3. Microscopy image of pandoravirus viruses together with various bacteria

The preparation observed was a mixture of pandoravirus viruses (a virion is indicated by a white arrow) with various bacteria (including *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escherichia coli*). Images were obtained with the Hitachi SU5000 instrument (Hitachi High-Technologies Corporation, Tokyo, Japan).

TABLES

Table 1. Timeline of some important discoveries regarding mimiviruses, their virophages, and other giant viruses of amoebae

Year, reference	Virus	Novelty
1992	Mimivirus	Isolation of the Mimivirus, considered a Gram positive coccus
2003 [1]	Mimivirus	First microorganism with a capsid and no ribosome
2004 [41]	Mimivirus genome	Largest viral genome and gene repertoire Translation components Hypothesis that giant viruses might represent a fourth domain of Life
2008 [42]	Marseillevirus	Giant viruses of amoebae are diverse
2009 [25]	Sputnik virophage	First virus of virus (of mimivirus)
2012 [34]	Pro-virophages and transpoviron	Expansion of the mimivirus mobilome
2013 [37]	Pandoraviruses	Giant viruses devoid of capsids and capsid-encoding genes
2012 [101]	Senegalvirus	First giant virus of amoebae isolated from a human
2013 [82]	Mimivirus LBA111	First mimivirus isolated from a human, a patient suffering from pneumonia
2013 [59]	Zamilon virophage	Some mimiviruses can resist to virophages
2015 [38]	Faustovirus	Giant viruses can infect amoebae other than *Vermamoeba vermiformis*
2016 [60]	Mimiviruses, Zamilon virophage	Mimiviruses can defend themselves against virophages through integration of short DNA fragments of these virophages: the MIMIVIRE system
2018 [5]	Tupanvirus	Largest set of translation components in the virosphere
2018 [17]	Tetraselmis virus 1	Genes encoding energy production (fermentation) enzymes in a mimivirus

Table 2. Contradiction by mimivirus and other giant viruses of criteria of definition of classical viruses

Definition criteria	Mimiviruses (and other giant viruses)
Virus size <0. 22 µm	Bigger (not ultrafilterable; visible by light microscopy)
Viruses contains only one type of nucleic acid, DNA or RNA	Viruses contain both (genomic DNA + messenger RNAs)
No binary multiplication	True
Parasitic lifestyle	True
Absence of energy metabolism enzymes	Two found in an alga-infecting mimivirus
Absence of components of the translation apparatus	Untrue (up to 70 and 20 tRNAs)
- no ribosomal proteins or genes	- True
Presence of a capsid	Absent in pandoraviruses; no known capsid morphology in some other giant viruses (pithoviruses, cedratviruses, orpheovirus)

Fig. 1

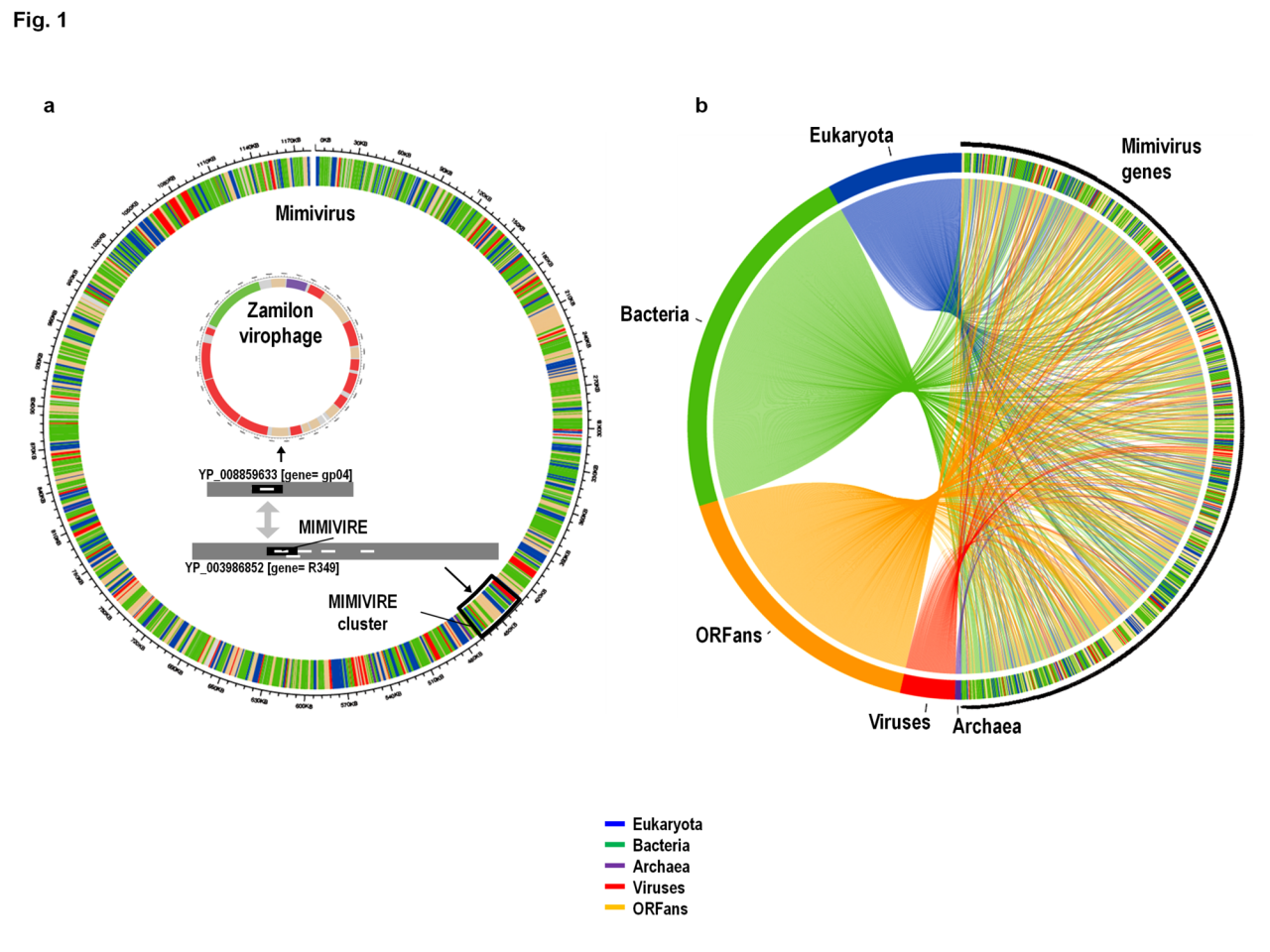
a
Mimivirus
Zamilon virophage
YP_008859633 [gene= gp04]
MIMIVIRE
YP_003986852 [gene= R349]
MIMIVIRE cluster
b
Eukaryota
Mimivirus genes
Bacteria
ORFans
Viruses
Archaea
Eukaryota
Bacteria
Archaea
Viruses
ORFans

Fig. 2

Mobile genetic elements, key players of mimivirus genetic evolution

Whereas classical viruses are considered as a mobilome component, giant viruses have their own mobilome [34], another feature that they share with cellular organisms [32].

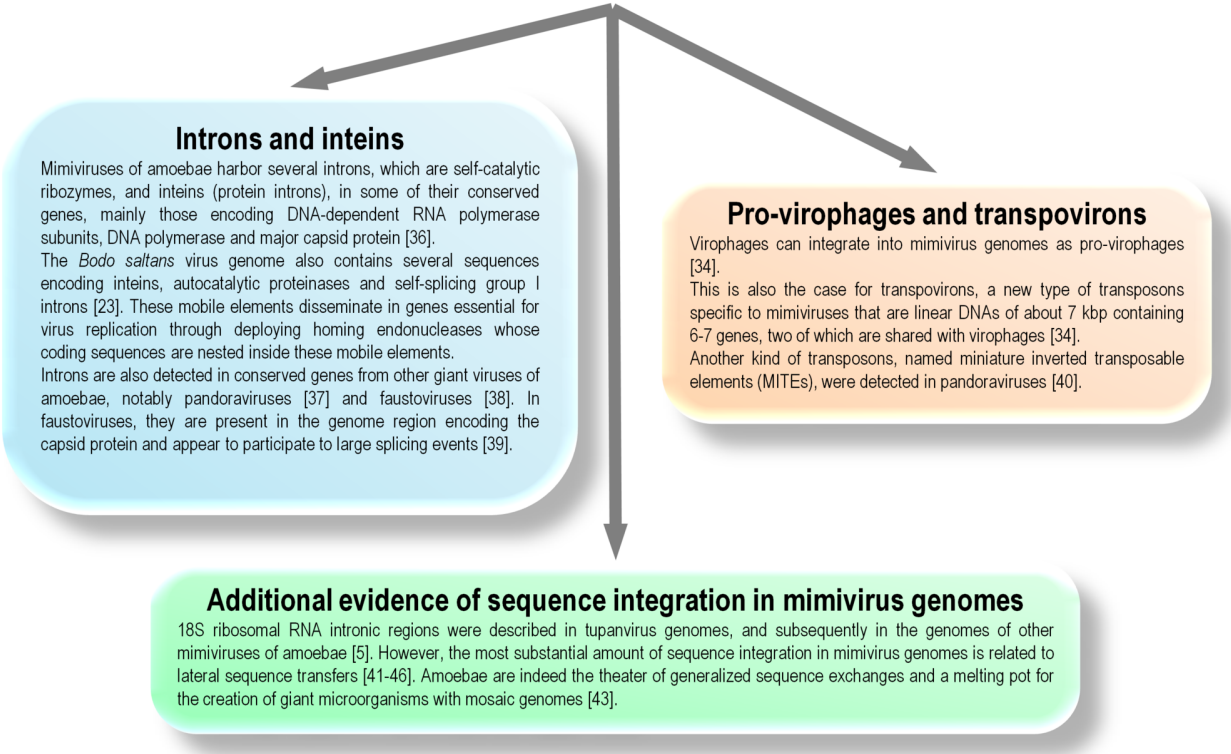

Fig. 3