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Giant mimiviruses escape many canonical criteria of the virus definition

P. Colson, Y. Ominami, A. Hisada, B. La Scola, Didier Raoult

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9 **Author list: Philippe COLSON¹, Yusuke OMINAMI², Akiko HISADA³, Bernard LA**
10 **SCOLA¹, Didier RAOULT¹ ***

11 **Affiliations:** ¹ Aix-Marseille Université, Institut de Recherche pour le Développement (IRD),
12 Assistance Publique - Hôpitaux de Marseille (AP-HM); Microbes, Evolution, Phylogeny and
13 Infection (MEΦI); Institut Hospitalo-Universitaire (IHU) - Méditerranée Infection, 19-21
14 boulevard Jean Moulin, 13005 Marseille, France.

15 ² Hitachi High-Technologies Corporation, Science & Medical Systems Business Group
16 24-14, Nishi-shimbashi 1-chome, Minato-ku, Tokyo 105-8717 Japan

17 ³ Hitachi, Ltd., Research & Development Group, 2520, Akanuma, Hatoyama, Saitama, 350-
18 0395 Japan

19 *** Corresponding author:** Didier RAOULT, IHU - Méditerranée Infection, 19-21 boulevard
20 Jean Moulin, 13005 Marseille, France. Tel.: +33 413 732 401, Fax: +33 413 732 402; email:
21 didier.raoult@gmail.com

22
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ABSTRACT

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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

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51 INTRODUCTION

52 Giant viruses of amoebae were discovered in 2003 (Table 1) [1]. The characterization of their
53 first representative, *Acanthamoeba polyphaga mimivirus* (APMV), prompted the quest for
54 other similar infectious agents. During the last 15 years, nine new groups of giant viruses
55 have been described [2]. They include marseilleviruses, pandoraviruses, pithoviruses,
56 faustoviruses, molliviruses, kaumoebaviruses, cedratviruses, pacmanviruses and
57 orpheoviruses [2,3]. Giant virus names have been chosen by their discoverers for various
58 reasons, due to specific features including morphological (as for the case of mimivirus, an
59 acronym for Mimicking Microbe Virus in memory of "Mimi the amoeba", example of the
60 story of the evolution told by his father to one of us (DR)), or to the geographical location of
61 virus isolation or discovery (as for the case of Marseillevirus isolated in Marseille, France)
62 [4]. Their particles have a size ranging between 200 and 2,300 nm and their genomes harbor
63 444-2,544 genes [2,5]. Thus, giant viruses are as big or even bigger than some bacteria and
64 they encode similar or greater number of genes. Beyond, data accumulated highlight that
65 there is a quantum leap between these infectious agents and classical viruses, and some
66 contain an almost comprehensive translation apparatus, although they lack ribosomes. In
67 addition, the first viruses of virus to be discovered, named virophages, were found to replicate
68 in mimivirus factories and their DNA can integrate into the genomes of mimiviruses, in
69 addition to mimivirus-specific transposons named transpovirons. Mimiviruses and virophages
70 strongly interact between each other, through sequence transfer, integration of virophages
71 DNA into giant virus DNA, and defense mechanisms of mimiviruses against virophages.
72 Therefore, giant viruses, virophages and their relationships represent new challenges in the
73 field of virology. We focused here on the widening gap between mimiviruses and other

74 viruses.

75 **MIMIVIRUSES**

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

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

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

106

107 **VIROPHAGES AND THE MIMIVIRUS MOBILOME**

108 The isolation of a second mimivirus in 2008 led to the discovery and description of a small
109 (≈ 50 nm-large) icosahedral virus (strain Sputnik), the first virus of virus [25]. It was named a
110 virophage by analogy with bacteriophages. Indeed, it was unable to multiply in the absence of
111 mimivirus, replicated in the mimivirus factories, slowed down the mimivirus replication and
112 was commonly associated with production of morphologically abnormal viruses. Sputnik
113 particles were found associated with mimivirus fibers [25-27]. Both virophages and
114 mimiviruses are produced within same viral factories. Sputnik appears to hijack the mimivirus
115 factory, being detected inside it and often produced before the giant virus or at a distinct
116 location. The 18,343 bp long circular dsDNA genome of Sputnik contains 21 genes including
117 14 ORFans and 7 ORFs with various putative origins including eukaryotic, bacterial, viral or
118 plasmidic (Figure 1). Such chimeric gene repertoire and the presence of putative integrases
119 and transposases led to hypothesize that this virophage might represent a vehicle of genes.
120 Another virophage, Mavirus (for Maverick virus) was isolated in association with CroV [28].
121 It also reduces amoebal cell lysis and production of its associated giant virus. Mavirus has a
122 19,063 bp circular dsDNA genome that encodes 20 proteins. Similar genome length and
123 structure and a close evolutionary relationship were noticed between mavirus and the self-

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

130 The mobilome represents all the kinds of mobile genetic elements, which are key
131 players of genetic evolution: it comprises transposable elements, plasmids, introns and inteins,
132 as well as viruses, [34]. The mimivirus specific mobilome corresponds to proviophages,
133 transpovirons, inteins and introns. Whereas classical viruses are considered as a mobilome
134 component, giant viruses have their own mobilome [34], another feature that they share with
135 cellular organisms [35] (Figure 2). Virophages are part of the mimivirus mobilome. They can
136 integrate into mimivirus genomes as pro-virophages [34]. This is also the case for
137 transpovirons, a new type of transposons specific to mimiviruses. They are composed of
138 linear DNAs of about 7 kbp containing 6-7 genes, two of which are shared with virophages
139 [34]. They depend on mimiviruses for their replication, being concentrated around the
140 mimivirus factories during the replicative cycle, and they accumulate in the amoebal
141 cytoplasm and the mimivirus and virophages virions.

142

143 **MIMIVIRUS SYMPATRIC LIFESTYLE IN AMOEBAE THAT ARE SITES OF** 144 **GENERALIZED SEQUENCE TRANSFERS**

145 The tremendous size and composition of giant viruses that infect protists have been linked to
146 their sympatric lifestyle, consisting in their survival and replication in a microbial community
147 [44]. The genomes of giant viruses are mosaics containing genes of eukaryotic, bacterial,
148 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 $\approx 16\%$) [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 Carroll's book *Through the looking-glass* where Alice runs as fast as she can with the
170 Red Queen through a chessboard, but nonetheless surprisingly 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

174 emerge. In the setting of continuous sequence transfers promoted by contacts between genetic
175 material from the intra-amoebal microorganisms during their multiplication or replication,
176 each microorganism may act as a tinkerer [57] by using sequence gains and losses to conceive
177 new attack or defense tools. Moreover, new adaptations may occur at the expense of previous
178 changes. This paradigm parallels that of the representation of genetic evolution as a rhizome,
179 which is devoid of a center and can grow through gaining or losing one or several elements
180 [49,58]. It can be illustrated by several recently described defense mechanisms involving
181 mimiviruses, including the defense of mimiviruses of amoebae against virophages.

182

183 **MIMIVIRUS AND VIROPHAGE DEFENSE MECHANISMS**

184 Following the observation that mimiviruses of group A, but not B-C, were resistant to the
185 Zamilon virophage [59], the means by which mimiviruses A could defend against this
186 virophage were investigated. This investigation was conducted by analogy with the defense
187 strategy of bacteria and archaea against viruses through CRISPR-Cas systems, which consists
188 in the integration into their genomes of fragments of these invaders' DNA ("cannibalism")
189 [60]. Thus, short specific Zamilon DNA fragments were searched for in the genomes of
190 mimiviruses A. Four copies of a 15 nucleotide-long Zamilon DNA fragment, named
191 MIMIVIRE for "mimivirus virophage resistance element", were detected within an operon
192 (Figure 1). This sequence was absent from the genomes of Zamilon-sensitive mimiviruses B-
193 C. The mechanism of this resistance might rely on nucleic acid sequence recognition and
194 confer immunity against virophages. The MIMIVIRE operon includes one gene that contains
195 the 15 nucleotide-long Zamilon sequences and two genes annotated as a helicase and a
196 nuclease, whose functions were experimentally validated. They may allow the cleavage of a
197 foreign nucleic acid. The restoration of mimiviruses A sensitivity to Zamilon virophage was
198 observed by independently inhibiting these three genes by RNA interference. Although it has

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

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

218

219 **THE GROWING MISMATCH BETWEEN GIANT VIRUSES AND THE CRITERIA** 220 **OF DEFINITION OF CLASSICAL VIRUSES**

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

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

228 The virus concept grew on the initial observations by Ivanowsky and Beijerinck that
229 infectious agents smaller than microorganisms, not ultrafilterable themselves, may exist and
230 transmit infections [67,68]. Congruently, these infectious agents were not visible by light
231 microscopy that allows seeing shapes larger than 200 μm . This paradigm was applied to
232 viruses until the APMV discovery (Table 2). The modern concept of virus was delineated in
233 1957 by Lwoff [69], although intracellular bacteria and viruses were grouped together in
234 microbiology books until the 1970s. Lwoff stated that viruses contain only one type of nucleic
235 acid, DNA or RNA; reproduce only from their genetic material, being unable to grow and
236 divide; and do not harbor in their genomes information encoding an energy production
237 system. The viral capsid was described a few years later [70]. Messenger RNAs are in fact
238 detected in giant viruses, many having been detected in APMV particles [41]. Therefore,
239 these viruses contain both DNA and RNA. While giant viruses do not multiply through binary
240 fission, those with ovoid viruses (including pandoraviruses, pithoviruses and cedratviruses)
241 have a weird assembly stage during which the viruses tegument seems to grow progressively
242 while enclosing gradually its content [37]. Moreover, giant viral factories look like morula
243 from *Chlamydia* spp. [71]. The emblematic lack of enzymes involved in intermediate
244 metabolism was recently challenged by the finding in a green alga-infecting mimivirus named
245 Tetraselmis virus 1 of two key fermentation genes encoding a pyruvate formate-lyase and a
246 pyruvate formate-lyase activating enzyme [17]. Besides, some obligatory intracellular
247 parasites among which the small bacteria *Carsonella ruddii* are devoid of a complete ATP
248 generation machinery [72]. Regarding the capsid, mimiviruses, as a majority of giant viruses,

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

253 Furthermore, emblematic elements that are lacking among classical viruses are protein
254 synthesis components. Ten translation-related genes were described in the APMV genome
255 [41]. They corresponded to aminoacyl-transfer RNA (tRNA) synthetases or translation
256 factors. In addition, six tRNA-encoding genes were present. It was later shown that RNA
257 silencing of the gene encoding a putative translation initiation factor (R458) was associated
258 with a prolonged eclipse phase and a delay in the appearance of the mimivirus factory, along
259 with modulation of expression of 32 mimivirus genes [75]. Recently, the mimivirus
260 translation component set showed a dramatic expansion with the assembly of genomes of
261 putative non-isolated mimivirus relatives, named klosneuviruses [22], then with the *Bodo*
262 *saltans* virus discovery [23]. Nevertheless, current record holders are tupanviruses whose
263 genomes harbor the most complete set of translation-related genes of the virosphere, with all
264 20 aminoacyl-tRNA synthetases, and 67-70 tRNAs associated with 46-47 codons [5]. In
265 addition, there are two different copies of an intronic region of 18S ribosomal RNA in the
266 tupanvirus genome, and such sequences were shown to be present in other mimiviruses of
267 amoebae. When excluding ribosomes, tupanvirus genomes harbor as many translation-related
268 genes as *Carsonella ruddii*, a bacterium, *Nanoarchaeum equitans*, an archaeon, and, in the
269 case of Tupanvirus deep ocean, *Encephalitozoon cuniculi*, an eukaryote. Thus, the major
270 cellular hallmark that still lacks in giant viruses is the ribosome, including both ribosomal
271 proteins and RNA-encoding genes. Overall, there is hence a quantum leap between classical
272 viruses and mimiviruses and other giant viruses that have a complexity similar to that of
273 intracellular microorganisms.

274

275 **THE PRESENCE AND SIGNIFICANCE OF GIANT VIRUSES IN HUMANS**

276 Beyond being currently considered as common in water and soils worldwide, giant viruses
277 have been detected in human samples in several studies and by several teams. Notably, hints
278 of their presence in humans have been increasingly reported through metagenomic analyses
279 [2,76-79]. This involved sequences related to mimiviruses and virophages but also to
280 marseilleviruses and more recently discovered giant viruses. Various other techniques have
281 contributed to identify the presence of giant viruses in humans, including serologies,
282 immunohistochemistry and immunofluorescence techniques, PCR and fluorescence *in situ*
283 hybridization (FISH) [2,80-84]. A critical step in studying the presence and significance of
284 giant viruses in humans was their isolation from human samples. A marseillevirus was in
285 2012 the first giant virus of amoebae to be isolated from a human, in the feces from a young
286 healthy Senegalese man [85]. Then, a mimivirus was isolated from the bronchoalveolar fluid
287 and the feces of two pneumonia patients [82,86], and from the urine of a kidney-transplant
288 recipient in whom no role of the giant virus could be speculated in the patient's symptoms that
289 consisted in acute diarrhoea and dyspnoea [9]. Based on serological assays, molecular tests
290 and culture isolation, mimiviruses were suspected to be linked to pneumonia [82], whereas
291 marseilleviruses were mostly detected in the blood and the lymphoid tissues and associated
292 with adenitis and Hodgkin lymphoma [83,84] . Besides, mice inoculated intracardially with
293 APMV developed histopathologically proven lesions of pneumonia [87] and a 30-day long
294 persistence of Marseillevirus but no disease was observed in rats and mice inoculated
295 parenterally, intraperitoneally or by aerosolization [88]. Overall, although the presence and
296 clinical significance of giant viruses in humans are still controversial issues [89,90], they
297 represent an emerging field that deserves broader analyses and increased consideration in
298 future studies.

299

300 **CONCLUSION**

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

309

310

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325

326 **Conflicts of interest**

327 The authors have no conflicts of interest to declare. Funding sources had no role in the design
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330

331

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BOXES

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Box 1. Main phenotypic and genotypic features of mimiviruses

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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".

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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].

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

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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].

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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.

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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.

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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].

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

FIGURE LEGENDS

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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).

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TABLES

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

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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-viropages 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 <i>Vermamoeba vermiformis</i>
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

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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 μ m	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)

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Fig. 1

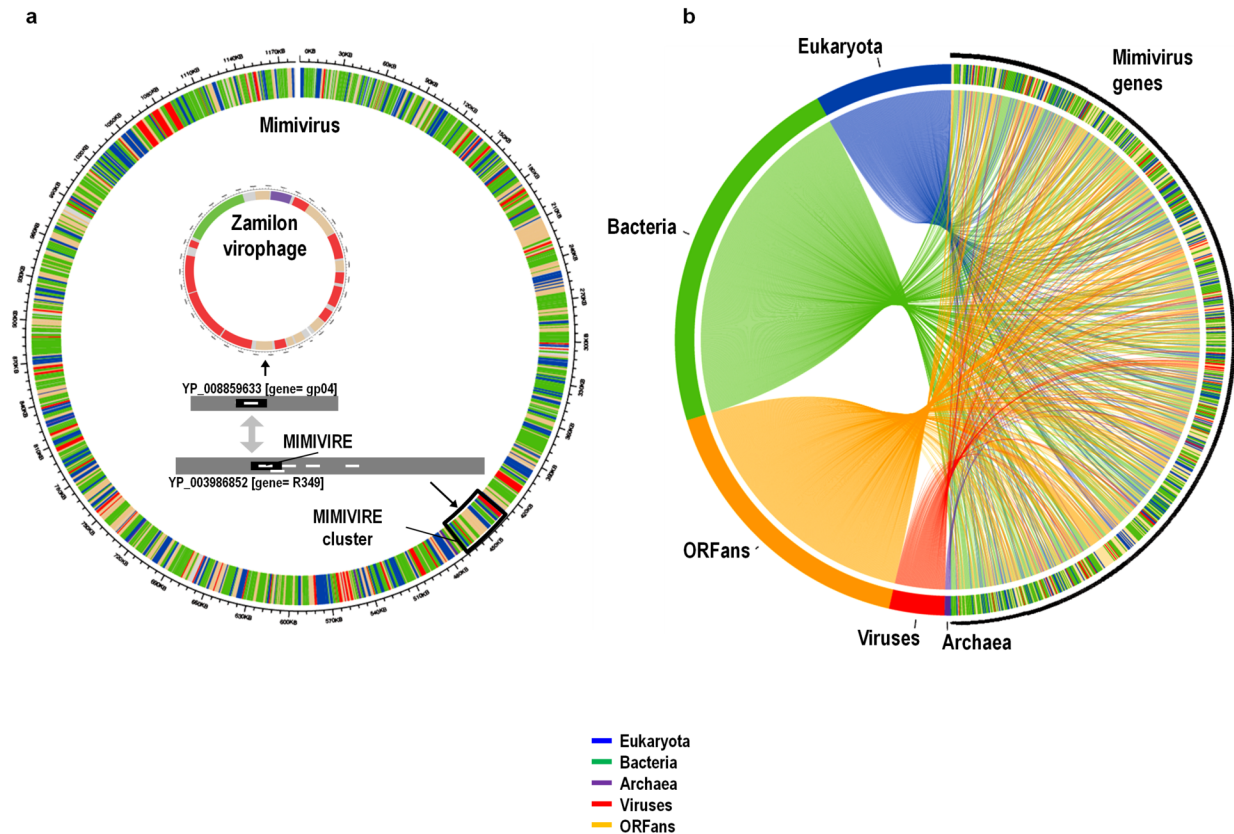


Fig. 2

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].

Introns and inteins

Mimiviruses of amoebae harbor several introns, which are self-catalytic ribozymes, and inteins (protein introns), in some of their conserved genes, mainly those encoding DNA-dependent RNA polymerase subunits, DNA polymerase and major capsid protein [36].

The *Bodo saltans* virus genome also contains several sequences encoding inteins, autocatalytic proteinases and self-splicing group I introns [23]. These mobile elements disseminate in genes essential for virus replication through deploying homing endonucleases whose coding sequences are nested inside these mobile elements.

Introns are also detected in conserved genes from other giant viruses of amoebae, notably pandoraviruses [37] and faustoviruses [38]. In faustoviruses, they are present in the genome region encoding the capsid protein and appear to participate to large splicing events [39].

Pro-virophages and transpovirons

Viropages can integrate into mimivirus genomes as pro-viropages [34].

This is also the case for transpovirons, a new type of transposons specific to mimiviruses that are linear DNAs of about 7 kbp containing 6-7 genes, two of which are shared with viropages [34].

Another kind of transposons, named miniature inverted transposable elements (MITEs), were detected in pandoraviruses [40].

Additional evidence of sequence integration in mimivirus genomes

18S ribosomal RNA intronic regions were described in tupanvirus genomes, and subsequently in the genomes of other mimiviruses of amoebae [5]. However, the most substantial amount of sequence integration in mimivirus genomes is related to lateral sequence transfers [41-46]. Amoebae are indeed the theater of generalized sequence exchanges and a melting pot for the creation of giant microorganisms with mosaic genomes [43].

Fig. 3

