Journal of Invertebrate Pathology 101 (2009) 172–180

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/yjipa

Mimivirus and Mimiviridae: Giant viruses with an increasing number of potential hosts, including corals and sponges

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A R T I C L E I N F O

Article history:
Received 14 January 2009
Accepted 6 March 2009
Available online 18 May 2009

Keywords:
Mimivirus
Mimiviridae
Nucleocytoplasmic Large DNA virus
Sponge
Coral

A B S T R A C T

Mimivirus, a giant DNA virus (i.e. "girus") infecting species of the genus *Acanthamoeba*, was first identified in 2003. With a particle size of 0.7 μm in diameter, and a genome size of 1.2 Mb encoding more than 900 proteins, it is the most complex virus described to date. Beyond its unusual size, the Mimivirus genome was found to contain the first viral homologues of many genes thought to be the trademark of cellular organisms, such as central components of the translation apparatus. These findings revived the debate on the origin of DNA viruses, and the role they might have played in the emergence of eukaryotes. Published and ongoing studies on Mimivirus continue to lead to unexpected findings concerning a variety of aspects, such as the structure of its particle, unique features of its replication cycle, or the distribution and abundance of Mimivirus relatives in the oceans. Following a summary of these recent findings, we present preliminary results suggesting that octocorals might have come in close contact with an ancestor of Mimivirus, and that modern sponges might be host to a yet unidentified, even larger, member of the Mimiviridae.

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1. Introduction

Mimivirus, a giant DNA virus infecting multiple species from the genus *Acanthamoeba*, was initially isolated in 1992, from a cooling tower in the West Yorkshire mill town of Bradford (England). Its discoverer, Timothy Rowbotham, the officer in charge of Britain's Public Health Laboratory Service, was investigating the possible source of a pneumonia outbreak, and was looking for legionella-like bacteria. Instead, he found a new microorganism resembling a small Gram-positive coccus that he was able to propagate within the laboratory amoeba *Acanthamoeba polyphaga*. Failing to recognize its viral nature, he baptized the new intracellular parasite "Bradfordcoccus", pending further characterization. Ten years later, La Scola et al. (2003) corrected the initial mistake, renaming this atypical "Microbe Mimicking virus" as "Mimivirus". The following year, the determination and analysis of the complete sequence of its 1.2 Mb genome revealed that Mimivirus was not simply exceptional by its size, but exhibited many unique features never yet encountered in a virus, most noticeably genes encoding central components of the protein translation machinery (Raoult et al., 2004; Abergel et al., 2007), previously thought to be the signature of cellular organisms. Since then, additional studies have led to more surprises such as the unmatched complexity (Renesto et al., 2006) of its particle and its unique DNA delivery system (Zauberman et al., 2008), a promoter exhibiting an unprecedented level of sequence conservation (Suhre et al., 2005), and the recent discovery of a new strain of Mimivirus naturally infected by a new kind of satellite virus called a "virophage" (La Scola et al., 2008; Ogata and Claverie, 2008; Claverie and Abergel, 2009). Altogether, these findings revived a number of speculations on the origin of large DNA viruses (also called "Giruses" to emphasize their unique properties (Claverie et al., 2006)), their potential role in the emergence of eukaryotes, as well as in the transition from RNA to DNA cellular genome (Claverie, 2006; Forterre, 2006). Exhibiting more genes than numerous bacteria (i.e. cellular microorganisms), Mimivirus is at odd with the traditional concept of "virus" essentially referring to the inert particle. Instead, the complex genome of large DNA viruses makes much more senses if we interpret it as the required blueprint for the construction of the complex "virus factory" they must recreate from scratch within their host upon each infection (Claverie et al., 2008; Claverie, 2006). These virus factories, resemble very much a transient "cell within a cell", importing the energy and metabolites it needs from the host cytoplasm. This new perspective makes the fundamental divide between viruses and parasitic cellular organisms (such as *Rickettsia*)

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0022-2011/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2009.03.011

much less absolute, suggesting that large DNA viruses might be the result of an alternative reductive evolution pathway toward intracellular parasitism along which the loss of protein translation, rather than other metabolic pathways, was the key event (Claverie, 2006). Focusing on the biology of the virus *within its host* rather than on the property of the virus *particle* naturally reinstall viruses into the realm of living "organisms" (Claverie, 2006; Pearson, 2008). Following the deciphering of Mimivirus' genome sequence, we are now engaged in the elucidation of the molecular details of its replication cycle in its *Acanthamoeba* host, an endeavor that is expected to generate more surprises as well as new insights into the evolutionary origin of giant viruses. In the meantime, other approaches, such as the sequencing of environmental DNA sample ("metagenomics"), or the partial sequencing of other large plankton viruses, have recently revealed the unexpected abundance of large DNA viruses related to Mimivirus within the marine environment (Ghedin and Claverie, 2005; Monier et al., 2008a). Many of these yet uncharacterized Mimiviridae are probably infecting a wide variety of marine protists. In this article, we now present additional findings suggesting that Mimivirus relatives may also infect widespread marine invertebrates such as corals (octocorallia) or calcareous sponges (*Petrobiona massiliana*, Porifera). It is quite ironical that the largest (and easiest to visualize) viruses, after escaping detection for so long, are now found so prominent in the aquatic world, where they probably have a significant ecological impact through their regulation of the planktonic populations, and the infection of ubiquitous marine invertebrates.

2. Mimivirus defines a new family of Nucleocytoplasmic Large DNA viruses (NCLDV)

In a landmark work of comparative genomics, Iyer et al. (2001) proposed to regroup a number of viruses with large double-stranded DNA genomes within the super-family of Nucleocytoplasmic Large DNA viruses (NCLDV). In this super-family they included the Phycodnaviruses (infecting algae), the Iridoviruses (mostly infecting fish and other animals), the Asfarviruses (agents of the african swine fever), and the most studied Poxviruses (infecting animals). Among the common features defining NCLDVs were a number of shared "core" genes, and a replicating cycle occurring mostly (if not entirely) within the host cytoplasm. Highly visible intra-cytoplasmic inclusions, called "virus factories", are thus a trademark of NCLDV infections. NCLDVs also exhibit a nicely symmetrical icosahedral particle (with the exception of Poxviruses),

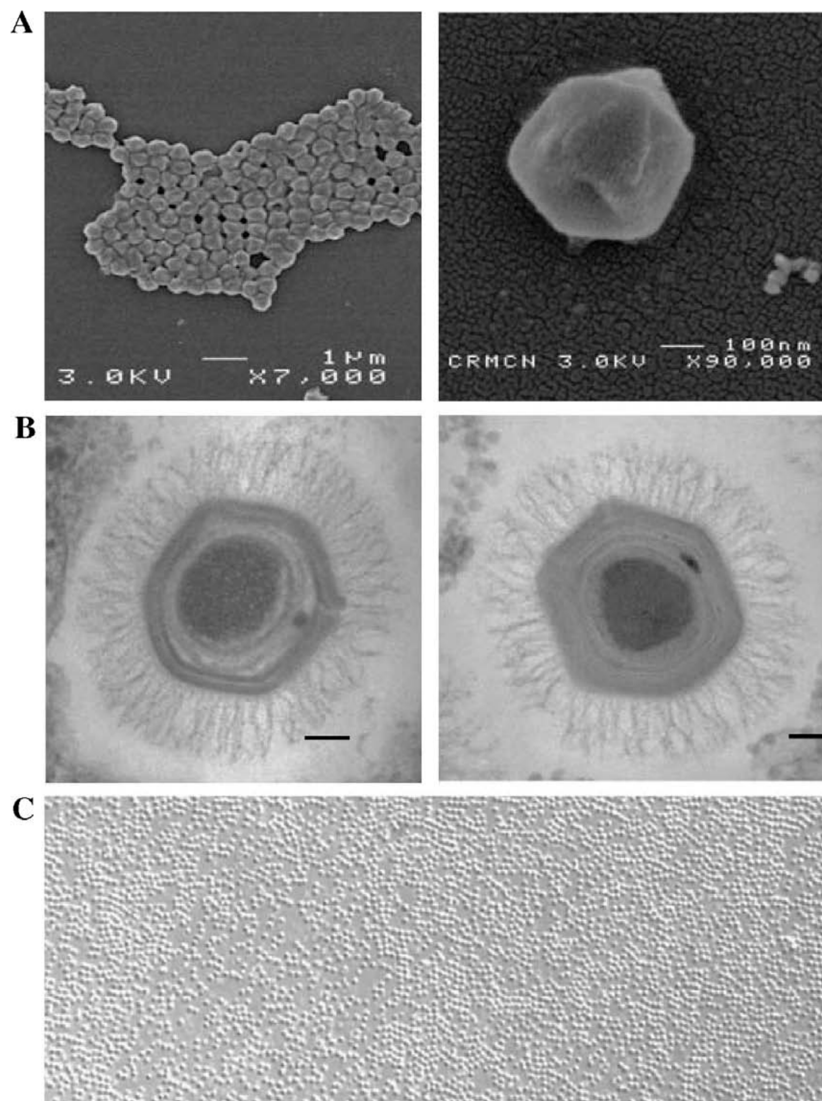

Fig. 1. Appearance of Mimivirus particles using different microscopy techniques. A: scanning electron microscopy; B: transmission electron microscopy of ultrathin sections, bar = 100 nm; and C: light microscopy (differential interference contrast, X63 Nomarski objective) of a carpet of purified particles deposited on a glass slide.

and a fairly complete virus-encoded transcription apparatus allowing them to replicate with minimal or no help from the host-encoded nuclear functions. Interestingly, the Chloroviruses (infecting the microalgae *Chlorella*), the most extensively studied Phycodnaviruses, do not have their own RNA polymerases. This is very likely the result of a lineage-specific loss, as closely related viruses with larger genomes, such as *Emiliania huxleyi* viruses (EhV, Wilson et al., 2005) exhibit their own transcription apparatus.

Despite their exceptional size (0.4–0.75 μm in diameter, depending on the visualization technique used, Fig. 1), the icosahedral shape of Mimivirus particles unmistakably suggests their viral nature (La Scola et al., 2003). The analysis of the Mimivirus 1.2 Mb genome sequence immediately indicated the presence of many of the previously defined NCLDV's core genes. Using a concatenation of the most conserved subset of them, Mimivirus was confidently positioned between the branch leading to the Phycodnaviruses (including Chloroviruses, EhV, and *Ectocarpus siliculosus* virus) and the one leading to the iridoviruses (such as Chilo iridescent virus, Regina ranaviruses, and Lymphocystis disease virus) (Claverie et al., 2008). Mimivirus thus defines a new family, the Mimiviridae, within the well established NCLDVs. Although Mimivirus exhibits a clear phylogenetic affinity with the above virus families, it appears closer to Poxviruses in term of its physiology, as we learn more about it. For instance, Mimivirus replication cycle appears to entirely take place in its host cytoplasm (Claverie et al., 2008) (Fig. 2), as expected from a Pox-like virus encoding a full transcription apparatus in its genome. The absence of early interactions with the nucleus of the infected host cell, implies that Mimivirus (like Poxvirus) particles must embark a functional – ready to go – transcription complex to initiate the transcription of the early genes in the host cytoplasm, immediately after infection (Resch et al., 2007). This was confirmed by our proteomic analysis of Mimivirus particles, where the protein products of most genes predicted to be involved in transcription were detected (Renesto et al., 2006).

Poxvirus (e.g. vaccinia virus) early class mRNAs are known to appear within minute after virus entry into the cell. This early transcription appears to occur within the confines of the core particle, that seems to retain much of its structural integrity after cell entry (reviewed in Broyles, 2003). In the case of Mimivirus, the inspection of the *Acanthamoeba* cytoplasm 2 h after infection consistently revealed small spherical compartments, precisely the size of the core of Mimivirus particles, the external layers of which remain visible in the phagocytic vacuoles (Fig. 3). It is thus tempting to propose that the transcription of the early Mimivirus genes, perfectly correlating with the presence of the highly conserved promoter element AAAATTGA (Table 1), takes place within these intra-cytoplasmic inclusions. Two hours post-infection, these particle-derived structures appear to function as "seeds" around which Mimivirus factories are initially formed, and then expand until they occupy most of the *Acanthamoeba* cell volume (Fig. 4).

In conclusion, the newly discovered Mimiviridae occupy a central position among the previously defined families of NCLDVs, exhibiting a phylogenetic proximity with phycodnaviruses and iridoviruses, as well as similarly symmetrical particles. On the other hand, Mimivirus is endowed with a full transcription apparatus making the early phase of its replication cycle independent from the host nucleus, and implying a poxvirus-like physiology. According to our preferred, if somewhat controversial, scenario of reductive evolution, the larger genome of the Mimiviridae might have retained more features of an hypothetical common ancestor than any of the other extant NCLDV families. We could picture this ancestral NCLDV, with an icosahedral particle and a full transcription apparatus, carrying a superset of the extant NCLDVs genes. The differences between various NCLDV families (Phycodnaviruses, Asfarviruses, Poxviruses, Iridoviruses and Mimiviruses) would be due to numerous lineages-specific gene losses. Such a scenario was well documented for intracellular parasitic bacteria (e.g. *Rickettsia*, *Borrelia*) (Blanc et al., 2007; Lescot et al., 2008). Following the same line of thought, some of Mimivirus unique features, such as the presence of several central translation components

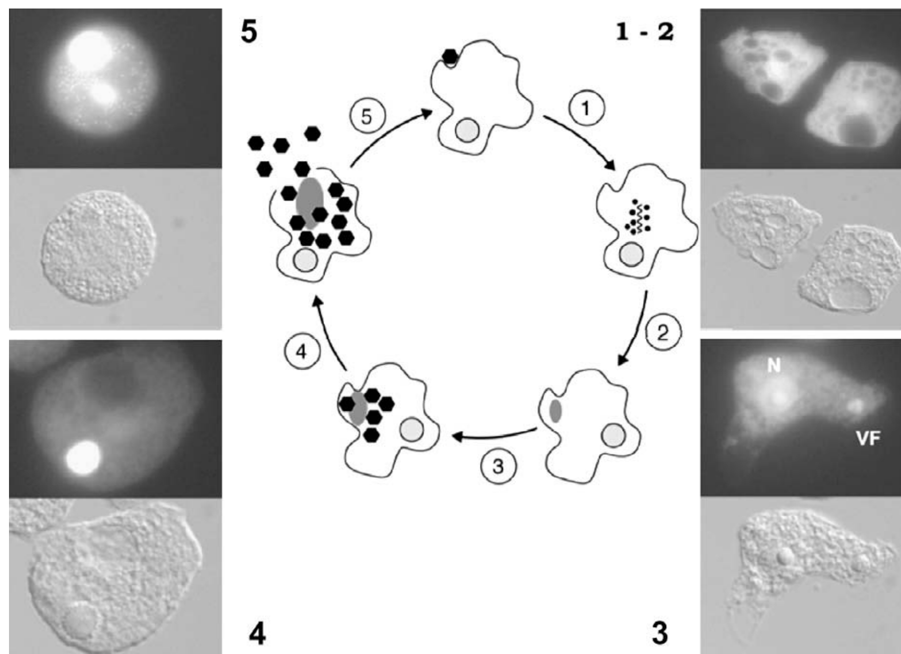

Fig. 2. Replicative cycle of Mimivirus in *Acanthamoeba castellanii*. Observation of infected cell at various time after infection, using fluorescence and Nomarski microscopy (X63 objective). 1 and 2: from 0 to 3 h post infection (no visible sign of infection); 2 and 3: 3–4 h after infection: apparition of a bright/contrasted "virus factory" (VF) distant from the cell nucleus (N); and 4: 4 h after infection: the virus factory increases in size and become much brighter/contrasted than the cell nucleus; 4–6 h post infection: The initial virus factory increases further in size and brightness, some secondary factories appear, numerous particles become visible in the cytoplasm.

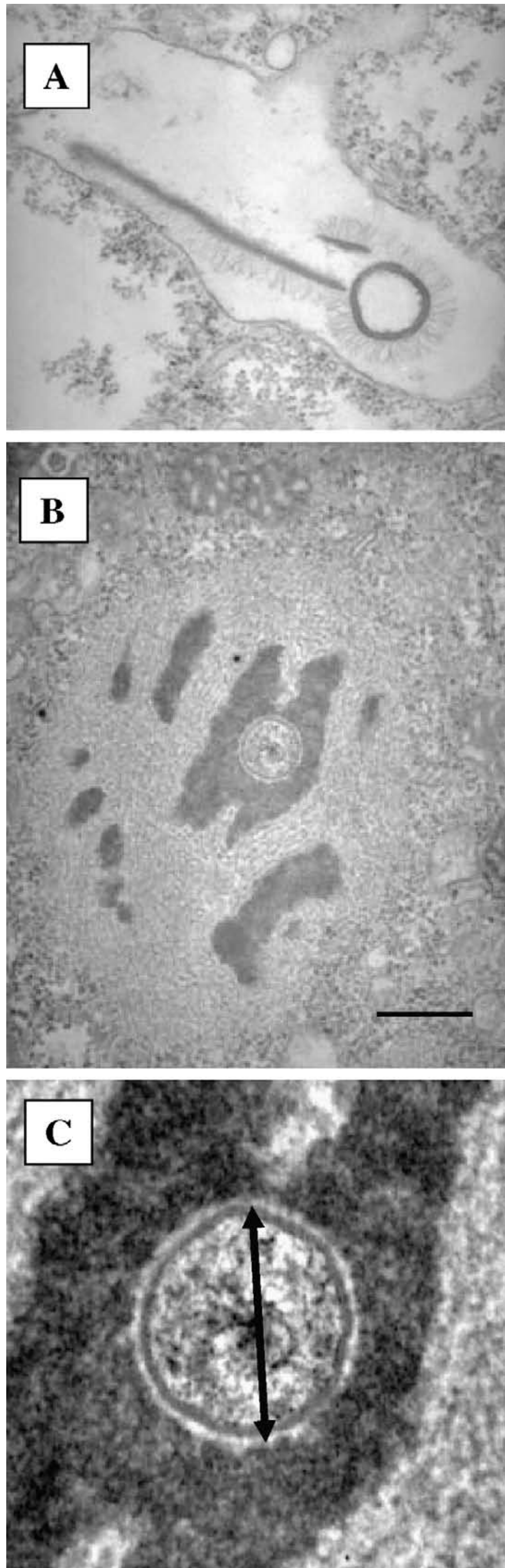

Fig. 3. Early phase of Mimivirus infection. (A) Empty "hairy" particles, sometimes unfolded, remaining in a phagocytic vacuole; (B) early stage of virus factory formation, seeded by a single "spherical inclusion" (bar = 500 nm); and (C) the diameter of the spherical inclusion corresponds to the one of Mimivirus "particle core" (the inner circle, 0.34 μm in diameter) (see Fig. 1). The morphology and thickness of the layers (10 nm for the white one, 12 nm for the dark one) delimiting these inclusions are not compatible with a standard lipid membrane.

(e.g. aminoacyl-tRNA synthetases), may even suggest that this NCLDV ancestor could have been harboring a more complete (even fully functional?) translation apparatus. The finding of an isoleucine-tRNA synthetase (as well as of other unusual genes uniquely found in Mimivirus) in the partially sequenced 730 kb-genome of the marine heterotrophic nanoflagellate *Cafetaria Roenbergensis* virus (CroV, M. Fischer, M. Allen, W. Wilson, C. Suttle, 5th Aquatic virus workshop, Vancouver, 6–11 July, 2008) is very much in favor of an ancestral NCLDV endowed with many, if not all, basic cellular functions (Claverie and Abergel, 2009).

3. Mimiviridae are very abundant in aquatic (marine) environments

Soon after the determination of the full genome sequence of Mimivirus, Ghedin and Claverie (2005) looked for the presence of related viruses in marine environments by running an exhaustive comparison of the predicted Mimivirus protein sequences with the "Sargasso Sea" metagenomic data set. This database of environmental sequences was generated in the first phase of the Global Ocean Sampling Expedition (GOS) at the initiative of C. Venter (Rusch et al., 2007). Among the 911 Mimivirus ORFs, Ghedin and Claverie (2005) found that 138 (15%) exhibited their closest match (with Blastp E-values ranging from 10^{-74} to 10^{-4}) to environmental sequences, and vice versa (i.e. reciprocal best matches). Moreover, 43% of the Mimivirus core genes (i.e. shared with other NCLDVs) were found to have their closest homologues in the Sargasso Sea database. This first study solidly established the existence of numerous uncharacterized Mimiviridae in the marine environment, albeit without providing a quantitative assessment of their prevalence.

More recently, Monier et al. (2008a) used a new "phylogenetic mapping" approach to analyze the largely increased GOS data set in a more quantitative manner. Briefly, they first established a global phylogenetic tree encompassing all known types of DNA polymerase sequences (a key enzyme shared by large DNA viruses and cellular organisms) and mapped each environmental sequence homologous to a DNA polymerase to its best fitting branch. First, this analysis confirmed that bacteriophages were the most abundant among marine viruses, validating the methodology. It then indicated that marine viruses related to the Mimiviridae were the second most abundant group, largely dominating the other known eukaryotic viruses, including Phycodnaviruses (Monier et al., 2008a). These yet uncharacterized Mimiviridae probably infect a variety of heterotrophic or mixotrophic (phagotrophic) protists ("grazers"), that have yet to be identified. Supporting this global analysis, Monier et al. (2008b) also noticed the best sequence similarity of Mimivirus DNA polymerase with the ones of three other large DNA virus infecting three different microalgal species: the haptophytes *Chrysochromulina ericina* (CeV01 with a genome size of 510 kb) and *Phaeocystis pouchetii*, (PpV01, 485 kb), and the prasinophyte (green algae) *Pyramimonas orientalis* (PoV01, 560 kb). In summary, although most of them have yet to be isolated and characterized, marine Mimiviridae already appear as a significant component in the ecology of micro- and pico-plankton populations, throughout the oceans (and probably in fresh water environments too).

4. MutS: a clear, yet mysterious, link between Mimiviridae and corals

In the previous section, we presented the growing evidence suggesting that yet elusive Mimiviridae represent a significant fraction of the viruses found in the oceans, most likely infecting a wide variety of plankton species. However, more indirect evidence

Table 1
Correlation of the AAAATTGA promoter element with early expressed genes.

T	10 Most expressed	20 Most expressed	30 Most expressed	40 Most expressed	50 Most expressed	60 Most expressed	70 Most expressed	80 Most expressed	90 Most expressed	100 Most expressed
30 min	10	20	30	38	48	56	64	74	84	93
3 h	8	13	21	28	37	45	53	57	65	72
6 h	2	2	2	2	2	2	3	3	4	5
9 h	1	2	2	2	2	3	3	5	6	10

For each time-point post-infection, the level of expression of each Mimivirus gene was ranked according to the number of 454-Flex tags mapping to the corresponding ORF. About 70.000 usable tags were generated for each time point (Byrne et al., 2009). The fraction of genes associated to the AAAATTGA motif was computed for the most expressed genes and clustered in groups of ten in descending order. Each cell indicates the number genes exhibiting the AAAATTGA motif. The presence of the motif is strongly positively associated with genes highly expressed immediately after infection. In contrast, the motif is strongly avoided for genes highly expressed after $T = 6$ h.

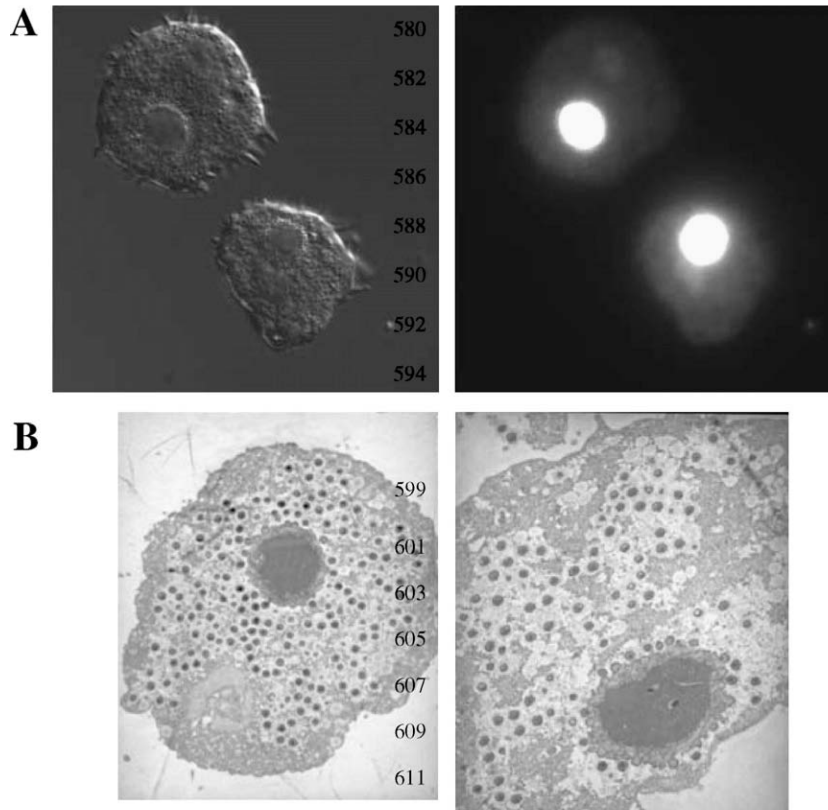

Fig. 4. Mature Mimivirus factories in *A. castellanii*. (A) Mimivirus factory in full production in two infected cells (left: Nomarski objective, right: fluorescence after DAPI staining); (B) new Mimivirus particles surrounding a virus factory (dark circle) (electron microscopy on ultra thin sections).

are also linking Mimivirus to ubiquitous marine invertebrates, such as corals and sponges.

In 1995, the serendipitous finding that the mitochondrial DNA of the soft coral *Sarcophyton glaucum* (phylum *Cnidaria*, class *Anthozoa*, subclass *Octocorallia*, order *Alcyonacea*) contained a gene clearly homologous to a bacterial MutS was reported (Pont-Kingdon et al., 1995). The presence of a putative mismatch repair gene in the mitochondrial DNA was then shown to be a unique feature of all *Octocorallia*, including species from the three main orders *Alcyoncea*, *Gorgonacea*, and *Pennatulacea*, and not found in any other mitochondrial genomes, including those of the sister group *Hexacorallia* (Brugler and France, 2007, 2008).

The mismatch-recognition protein MutS is one of the three components of an ubiquitous cellular DNA repair system that also involves a nicking endonuclease (MutH) and MutL that makes a bridge between MutS and MutH (reviewed by Malik and Henikoff, 2000). The simple level of sequence similarities, as well as molecular phylogenetic analyses, indicate the unambiguous relationship of the *Octocorallia* MutS homologue with a bacterial MutS gene uniquely found in different bacteria (e.g. Sulfurimonas, Nitratiruptor, Arcobacter) of the epsilon division (Fig. 5). This strongly suggests, as the most parsimonious scenario, that the *Octocorallia* ancestor acquired its mitochondrial MutS gene by lateral gene transfer, from an ancestor related to one of the above bacteria. Sulfurimonas and Nitratiruptor are chemoautotrophic bacteria, most often found in deep sea hydrothermal fields, where vestimentiferan tubeworms are also found. These worms lack digestive organs but harbor chemoautotrophic bacteria in a specialized tissue, the trophosome. Interestingly, the non chemosynthetic Arcobacter also exists as an intracellular endosymbiont, in the vestimentiferan trophosomes. The highly similar version of MutS shared by these three different bacteria, is quite divergent from the one found in the rest of the epsilon division. This suggests that a lateral gene transfer probably occurred between these bacteria sharing the same biotope. Interestingly, Arcobacter, was also isolated from the surface of infected corals (Frias-Lopez et al., 2002).

The sequencing of the Mimivirus genome revealed the presence of many genes previously never found in a virus. One of them was

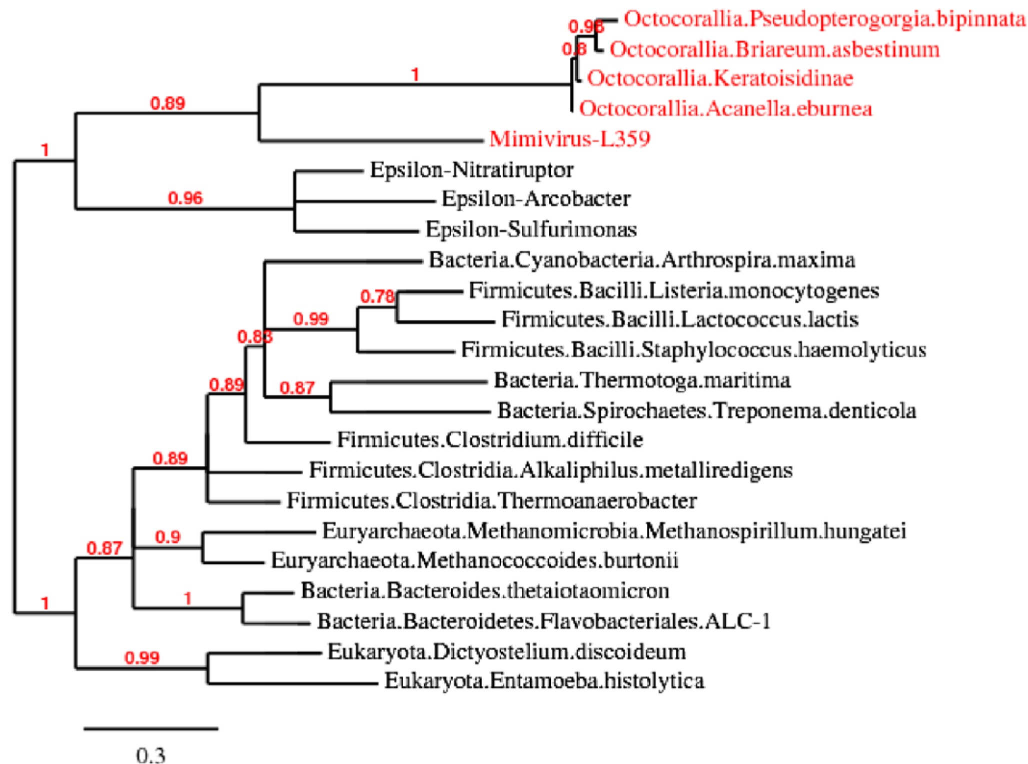

Fig. 5. Phylogenetic tree of the MutS homologues found in the three domains of life (Eukaryota, Eubacteria, and Archaea). The MutS proteins encoded by the mitochondrial DNA of various Octocorallia species (in red) and the Mimivirus L359 ORF (in red) are forming a cluster of their own, along side of the MutS proteins from three specific bacteria from the Epsilon division. All MutS proteins from other bacteria, eukaryotes, and archaea, are found in a different cluster. Computations were made using the "one click" default option of the Phylogeny.fr server (Dereeper et al., 2008).

the first virally encoded MutS homologue. This finding became even more puzzling when we realized that it was the same MutS version uniquely found in the *Octocorallia* mitochondrial genomes. The Mimivirus L359 ORF shares 31% identical residues (*P*-value < *E*−116) with the Nitratiruptor protein sequence, and 25% identical residues (*P*-value < *E*−65) with the MutS homologues of *Octocorralia* (from order *Pennatulacea*, *Gorgonacea* or *Alcyoncea*) over their entire lengths, close to 1000 residues. On the other hand, the Nitratiruptor MutS sequences shares 50% of identical residues with its closest bacterial homologues (Arcobacter), and 26% identical residues with its closest eukaryote homologue, the mitochondrial MutS of *Renilla reniformis* (*Octocorallia*: *Pennatulacea*). The Mimivirus MutS homologue is thus approximately equidistant from the bacterial and the *Octocorallia* MutS sequences, a position suggesting that an ancestral Mimiviridae might have been the vector of the bacterial MutS gene into the *Octocorallia* mitochondrial genome. In this context, it is worth to remember that Amoeba (or other phagocytic cells), in concentrating various bacterial species as food or endosymbionts, have been proposed to act as "melting pots" of genetic material and provide ample opportunities for horizontal gene transfers between water-borne bacteria or viruses (Ogata et al., 2006). Accordingly, giant viruses infecting amoeba could then be central to the lateral transfers of genes from the prokaryotic to the eukaryotic domain, or vice versa. Confirming the pattern of sequence similarity, a phylogenetic reconstruction nicely positions the Mimivirus sequence in between the Nitratiruptor/Sulfurimonas/Arcobacter group and the *Octocorallia* group.

The common origin of these various MutS sequences is further confirmed by the unique structure they are sharing, distinct from all other known mismatch-recognition proteins. As previously noticed by Malik and Henikoff (2000), the *Octocorallia* MutS is fused with a C-terminal HNH nicking endonuclease domain, allowing it to function in absence of a MutL homologue. Confirming our horizontal gene transfer scenario, this C-terminal HNH domain is also present in the Mimivirus L359 ORF, and in the Nitratiruptor/Sulfurimonas/Arcobacter genes (Fig. 6).

Last, but not least, the partial sequencing of several large NCLDVs phylogenetically related to Mimivirus (such as the previously cited CroV, CeV01, PpV01, PoV, as well as HcV, a virus infecting the dinoflagellate *Heterocapsa circularisquama*) indicated that they all exhibit a MutS homologue (Ogata et al., unpublished results). This again suggests their common ancestry or, alternatively, a large frequency of gene exchanges between these viruses.

In conclusion, we propose that an ancestral Mimiviridae played a central role in ferrying the MutS gene into the *Octocorallia* mitochondrial genome, before the radiation of the *Octacorallia* (450 My ago, perhaps before) into the three main orders *Alcyonacea*, *Pennatulacea*, and *Gorgonacea*, but after the divergence of the *Octocorallia*/*Hexacorallia* sister groups, as no trace of MutS gene is found in the mitochondrion of *Hexacorallia* species. To play this role, this ancestral Mimiviridae had to exist in close proximity of corals or, even better, be able to infect coral tissues. Coral cells with phagocytic properties have been described, and would be the primary target for such a process. We are now testing this hypothesis by looking for sign of NCLDVs near or within corals.

5. Putative Mimiviridae infection in a calcareous sponges (*Petrobiona massiliana*, Phylum Porifera, Order *Baeriida*)

In the early sixties, Jean Vacelet was pioneering the study of the calcareous sponge *P. massiliana*, endemic to the Mediterranean waters, and collected off the coast of Marseilles, France. In his PhD thesis (Vacelet, 1964), devoted to the anatomy and histology of *P.*

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Renilla.ren (898-964)      PSKYNSKVFIDSCEICGAPAK-------AIHHIKPKKSR----------S
Leptogorgia (900-966)      PSKYNAQVFIDSCEICGAPAE-------AIHHIQPKKLR----------N
Sarcophyton (893-963)      PSKYNAQVFIDSCEICGAPAD-------AVHHIKPKSEHKKLC------N
MIMIVIRUS  (979-1062)      KSNYNKDLLVKQCMICRYIPTTEYHKELESHHIHFQKNCWTDGKIKEKPY
Epsilon.Nit (888-960)      RSRYNKGVYLTKCAICNEVVE-------EVHHIEPKAKA-KGGFID---H
Epsilon.Arc (906-979)      TSKYNNSVFASNCVICGRACD-------DVHHIKEQARANKDGFIG---H
Epsilon.Sul (893-966)      SSKYNKELYVTKCVICGDMAE-------DVHHIKHKSLADEDGFIG---H
                            *.**  :   .* **              ***  :

Renilla.ren (898-964)      FNLNRKYNLVPVCSNCHLDIHRNKISILGWKKTP
Leptogorgia (900-966)      RKLNRRSNLVPVCSSCHLDIHKNKISILGWKRTP
Sarcophyton (893-963)      RKLNRRSNLVPVCSSCHLDIHRNKISILGWKRTP
MIMIVIRUS  (979-1062)      LSKNKLYNLVVLCRKCHNKVHQGEIIINGYTDTT
Epsilon.Nit (888-960)      FKANHKFNLIPLCSKHHKMVHEGKLLINGFVMSD
Epsilon.Arc (906-979)      INANHKYNLIPLCKEHHKMVHDGTININGFVATS
Epsilon.Sul (893-966)      FHKNTKHNLIPLCKEHHKKIHEGKIRVDGFVMTS
                              *   **: :* .  *  :*  . : : *:  :
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Fig. 6. Extraneous HNH endonuclease domain. This domain is uniquely found at the C-terminal ends of related MutS homologues found in Mimivirus, three epsilon proteobacteria (Nit: *Nitratiruptor* sp. SB155-2, Arc: *Arcobacter butzleri*, Sul: *Sulfurimonas denitrificans*), and the mitochondrial genome of Octocorallia (*Renilla reniformis*: *Pennatulacea*, *Leptogorgia hebes: Gorgonacea*, *Sarcophyton glaucum: Alcyonacea*). The strictly conserved positions are highlighted in yellow, or in red when known to be part of the HNH endonuclease active site.

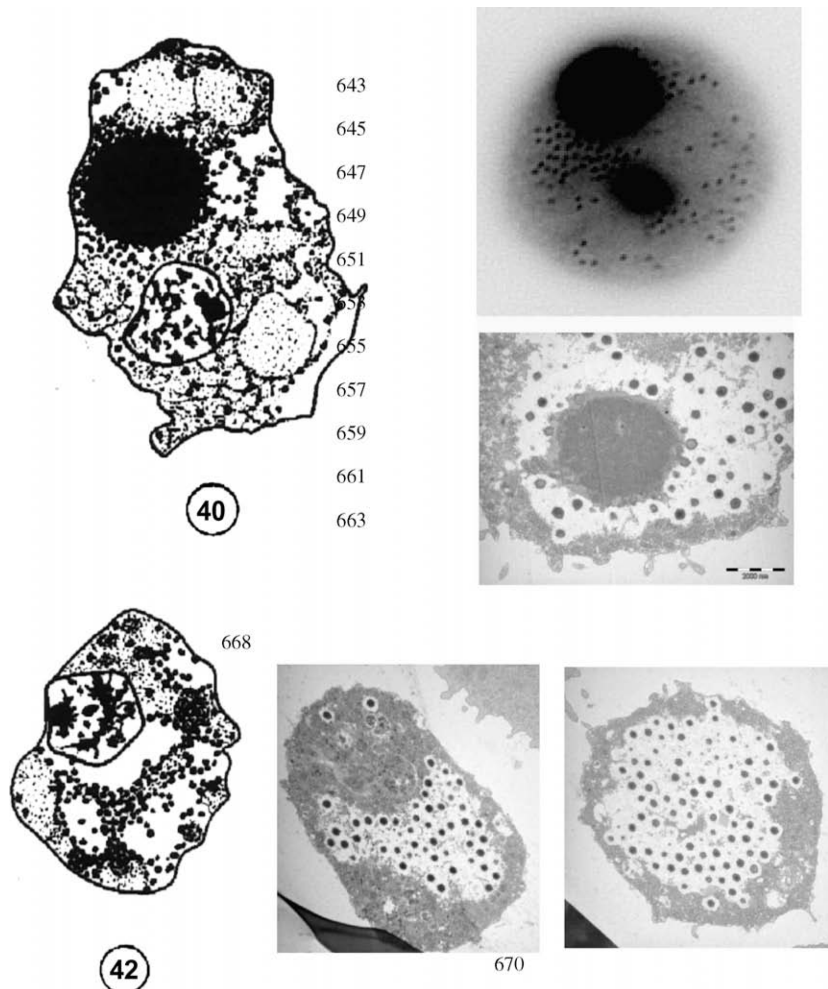

Fig. 7. Drawings of ''parasited'' sponge phagocytes (left, top and bottom, light microscopy after Bouin fixation and iron-hematoxylin staining, ×1000), strongly ressembling Mimivirus-infected *Acanthamoeba* cells (top right: fluorescence microscopy after DAPI staining (negative), others: electron microscopy of ultra thin sections). Drawings are reproduced from the PhD thesis (p. 27) of Jean Vacelet (Aix-Marseille University, June 8, 1964). The top illustration may correspond to a fully active virus factory starting to release new virus particles intracellularly. The bottom illustration may correspond to a later stage, just prior to the lysis of the host cell.

massiliana, he described the morphology of some large cells (15–50 μm in diameter), functionally similar to phagocytic cells called "megacytes" in other sponges (such as *Sycon raphanus*). While studying the origin and differentiation of these cells he called "phagocytes", in a number of specimens, he sporadically (in 15 individuals over 355) observed these cells undergoing a degeneration ultimately leading to cell death and lysis, with the release of numerous "granules". He then rightly suspected this to result from a "parasitic infection" of an unspecified nature, but did not study it further. Fig. 7 reproduces two illustrations of this phenomenon as drawn in its PhD thesis (p. 27, Fig. 40 and 42). The resemblance of these drawing with today's micrograph of an *Acanthamoeba* cell releasing Mimivirus particle from a very large and brightly fluorescent "virus factory" is striking. We now believe this was the observation of *P. massiliana* phagocytes undergoing an infection by a giant virus related to the Mimiviridae. Given the relative scale of the sponge phagocyte and *Acanthamoeba* pictures, the particles of this putative *P. massiliana* virus could be twice as large as Mimivirus' particles, hence its genome also considerably larger. We are now in the process of actively looking for such giant virus in *P. massiliana* specimens as well as in their immediate environment.

6. Discussion

Since the discovery of the first Mimiviridae, in a banal cooling tower in the West Yorkshire mill town of Bradford (England), indirect evidence (mostly from metagenomic studies) are steadily accumulating that this new family of giant viruses is ubiquitous in aquatic environments, and was probably overlooked as a significant regulator of planktonic populations. A phylogenetic analysis including both known viral DNA polymerase sequence and marine environmental sequences indicates that many viruses previously classified as "phycodnaviruses" are beginning to form a cluster around Mimivirus, distinct from another cluster including all the *Chlorella* viruses, *E. huxleyi* virus, *Heterosigma akashiwo* virus1 and other microalgal viruses (Monier et al., 2008a,b). It seems only a matter of time before Mimiviridae could be definitely recognized as a separate, quantitatively important, group of aquatic viruses.

These yet uncharacterized *Mimiviridiae* seem to thrive in the aquatic environment, where we hypothesize that they use the size of their particle (larger than 200 nm in diameter for the core particle, eventually increased by the presence an external fiber layer of 100 nm or more) to mimic the organic debris, or small bacteria that are the normal food of a wide variety of heterotrophic (or mixotrophic) marine protists such as the Haptophytes (*Phaeocystis* and *Chrysochromulina* viruses), the *Prasinophytes* (*Pyraminomas* viruses), dinoflagellates (*H. circularisquama* virus), nanoflagellates (phylum *Chromista*) (*Cafeteria roenbergensis* viruses), or *Acanthamoeba* (Mimivirus). Although phylogenetically diverse, these virus hosts are all endowed with phagocytosis, probably the common route of infection for all Mimiviridae.

We believe that such a generic feeding-like mode of infection, dispenses these viruses from evolving a highly specific interaction with a host surface receptor, and is key in explaining how a group (probably monophyletic) of strongly related viruses, all with large genomes and particle sizes, have evolved to infect such a taxonomically wide variety of different micro-organisms. It is also likely that the uniquely complex Mimiviridae genomes, and their expression in well-delimited virus factories, are making these giant viruses quite independent from the physiological details of their hosts, within which only the most basic and conserved cellular machinery (e.g. the translation apparatus) is "hijacked".

This paradoxical host flexibility was already exemplified by Mimivirus both able to infect and replicate in a number of *Acanthamoeba* species (we tested environmental isolates of *A. polyphaga*, *Acanthamoeba castellanii*, *Acanthamoeba griffini*, *Acanthamoeba lenticulata*, *Acanthamoeba quina*, Abergel et al., unpublished results), as well as to penetrate phagocytes of mammalian origin, although the actual virus production in these cells is still debated (Ghigo et al., 2008; Dare et al., 2008; Larcher et al., 2006).

Following this line of thought a little further, one may then expect that some Mimiviridae could have evolved to infect marine animals that rely on environmental phagocytosis as a main nutrition system. We presented some preliminary evidence that this could be indeed the case for two phylogenetically distant marine invertebrates: *Octocorralia* and calcareous sponges. More are probably left to be discovered.

Acknowledgments

This project is funded by a grant from the French Research Agency ("ANR Blanc"). We acknowledge the use of the PACA-Bioinfo Bioinformatic platform (IBISA) and of the Phylogeny.fr server.

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