Giant viruses

Chantal Abergel, Jean-Michel Claverie

synthesis of the nucleotide second messenger cAAG in *Enterobacter cloacae* activates the effector Cap4, a promiscuous DNA endonuclease. Similarly, in *Escherichia coli*, a structurally distinct DNA endonuclease effector, NucC, is activated by the second messenger cAAA to degrade viral and bacterial genomic DNA to halt phage replication. Bioinformatic studies have revealed many CBASS operons encoding other Cap proteins with different putative effector functions including proteases, NADases, and potential pore-formation activities.

What are some open questions?

In vertebrates, direct recognition of DNA mislocalized to the cell cytosol is responsible for cGAS activation and initiation of 2′3′-cGAMP synthesis. However, how bacterial CD-NTases in CBASS immunity sense viral infection remains unknown. Bacteria lack organelles for sequestration of endogenous nucleic acid, indicating that if bacterial CD-NTase enzymes respond to viral DNA they must somehow sense unique ligands generated only during phage infection. Additionally, unlike human cGAS, many bacterial CD-NTases are constitutively active *in vitro*. Therefore, an alternative hypothesis is that CD-NTases may be held in a repressed state by endogenous metabolites, and activation occurs only when nutrients are rapidly consumed during viral replication.

Bacterial CBASS operons frequently contain additional *cap* genes of unknown function. For example, *V. cholerae* and *E. cloacae cap2* and *cap3* encode proteins with predicted homology to the eukaryotic E1/E2 ubiquitination machinery and ubiquitin-specific proteases. Likewise, structures of Cap proteins from *E. coli* and *Pseudomonas aeruginosa* CBASS operons demonstrate homology to eukaryotic HORMA-domain proteins, which are critical for DNA recombination and repair. In some instances, all *cap* genes within a CBASS operon are required for efficient defense, whereas in other cases, accessory *cap* genes beyond the effector protein appear dispensable for controlling phage infection. Understanding the functions of conserved *cap* genes is critical to reveal further insight into the mechanism of activation and regulation of CD-NTase signaling.

Finally, a major open question in CD-NTase biology is: what is the function of diverse unexplored CD-NTase enzymes encoded in animal genomes? In addition to cGAS, CD-NTase-family members encoded in the human genome include predicted enzymes like MB21D2 and Mab21-family proteins. Mutations in these genes are implicated in oncogenesis and as a cause of developmental disorders, suggesting the existence of diverse functions for CD-NTase signaling pathways beyond antiviral immunity.

Where can I find out more?

Ablasser, A., and Chen, Z.J. (2019). cGAS in action: Expanding roles in immunity and inflammation. Science *363*, eaat8657.

Burroughs, A.M., Zhang, D., Schaffer, D.E., Iyer, L.M., and Aravind, L. (2015). Comparative genomic analyses reveal a vast, novel network of nucleotide-centric systems in biological conflicts, immunity and signaling. Nucleic Acids Res. *43*, 10633–10654.

Cohen, D., Melamed, S., Millman, A., Shulman, G., Oppenheimer-Shaanan, Y., Kacen, A., Doron, S., Amitai, G., and Sorek, R. (2019). Cyclic GMP-AMP signalling protects bacteria against viral infection. Nature *574*, 691–695.

Kranzusch, P.J. (2019). cGAS and CD-NTase enzymes: structure, mechanism, and evolution. Curr. Opin. Struct. Biol. *59*, 178–187.

Lau, R.K., Ye, Q., Birkholz, E.A., Berg, K.R., Patel, L., Mathews, I.T., Watrous, J.D., Ego, K., Whiteley, A.T., Lowey, B., *et al*. (2020). Structure and mechanism of a cyclic trinucleotide-activated bacterial endonuclease mediating bacteriophage immunity. Mol. Cell *77*, 723–733.

Lowey, B., Whiteley, A.T., Keszei, A.F.A., Morehouse, B.R., Matthews, I.T., Antine, S.P., Cabrera, V.J., Kashin, D., Niemann, P., Jain, M., *et. al.* (2020). CBASS immunity uses CARF-related effectors to sense 3′–5′ and 2′–5′-linked cyclic oligonucleotide signals and protect bacteria from phage infection. Cell 182, 38–49.

Severin, G.B., Ramliden, M.S., Hawver, L.A., Wang, K., Pell, M.E., Kieninger, A.K., Khataokar, A., O'Hara, B.J., Behrmann, L.V., Neiditch, M.B., *et al.* (2018). Direct activation of a phospholipase by cyclic GMP-AMP in El Tor *Vibrio cholerae*. Proc. Natl. Acad. Sci. USA *115*, E6048–E6055.

Whiteley, A.T., Eaglesham, J.B., de Oliveira Mann, C.C., Morehouse, B.R., Lowey, B., Nieminen, E.A., Danilchanka, O., King, D.S., Lee, A.S.Y., Mekalanos, J.J., *et al.* (2019). Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. Nature *567*, 194–199.

Ye, Q., Lau, R.K., Mathews, I.T., Birkholz, E.A., Watrous, J.D., Azimi, C.S., Pogliano, J., Jain, M., and Corbett, K.D. (2020). HORMA domain proteins and a Trip13-like ATPase regulate bacterial cGAS-like enzymes to mediate bacteriophage immunity. Mol. Cell *77*, 709–722.

[1]Department of Microbiology, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA 02115, USA. [3]Parker Institute for Cancer Immunotherapy at Dana-Farber Cancer Institute, Boston, MA 02115, USA.
*E-mail: philip_kranzusch@dfci.harvard.edu

Quick guide

Giant viruses

Chantal Abergel* and Jean-Michel Claverie

What are giant viruses? Giant viruses (Figure 1) are called 'giant' because they violate the property of 'fi lterability', which has classically been used to separate viruses from other microbes, ever since the isolation of the tobacco mosaic disease virus in 1892. The diverse families of giant viruses known today (pending others) were most likely overlooked because of the premature generalization that all viruses would be tiny enough to pass through standard sterilizing fi lters. In line with this belief, the viral nature of the fi rst giant virus — called mimivirus, prototype of the family Mimiviridae — was recognized only in 2003, after having been mistaken for a parasitic bacterium for more than a decade. Viruses are thus classifi ed as 'giant' based on the size (smallest dimension >250 nm) of their particles — the virion — making them visible by standard light microscopy. The mimivirus particle is an icosahedron of 450 nm in diameter, reaching 750 nm due to a layer of fibrils surrounding its capsids. Apart from the Mimiviridae, three other families of giant viruses have been described: the proposed Pandoraviridae, Pithoviridae and Molliviridae. They show different virion shapes: pandoraviruses and pithoviruses (1 to 2 μm in length, 500 nm in diameter) are oblate, whereas molliviruses are spherical (600 nm in diameter). These giant viruses also possess double-stranded DNA genomes, rivaling in sizes and gene contents those of cellular microbes: up to 1.6 million basepairs (Mb) for the mimiviruses, 2.7 Mb for the pandoraviruses and more than 600 kb for the pithoviruses and the molliviruses. Although there is no strict correlation between particle size and genome size for giant viruses, a genome larger than 400 thousand bases (kb) is sometimes used as an alternative threshold for defining giant viruses.

What do giant viruses infect? Mimivirus, the first giant virus, was isolated on *Acanthamoeba polyphaga*.

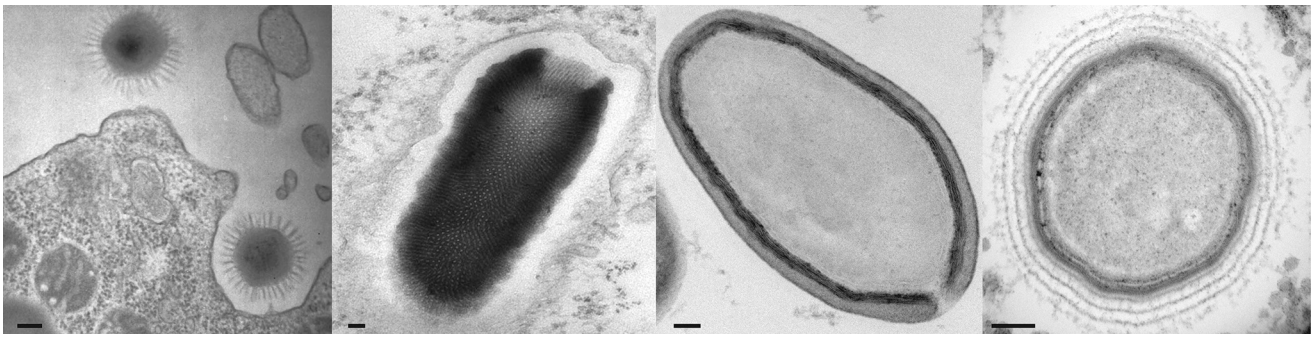

Figure 1. Giant viruses.
From left to right: mimiviruses (dsDNA genome up to 1.6 million bases; Mb), pithovirus (dsDNA genome up to 600 kb), pandoravirus (dsDNA genome up to 2.7 Mb) and mollivirus (dsDNA genome up to 600 kb). Scale bars: 100 nm.

These amoebae normally feed on bacteria and tolerate many of their prey as intracellular parasites. *Acanthamoeba castellanii* proved to be an excellent model system, as it led to the isolation of prototypes of three other families of giant viruses: the proposed Pandoraviridae, Pithoviridae and Molliviridae. During this research, new virus types with smaller particles and less complex genomes were also discovered: the Marseilleviridae, the Asfarviridae-related faustoviruses and pacmanviruses, and most recently, medusavirus. Members of the mimiviruses were also found to infect diverse microalgae (haptophytes and Chlorophytes), extending the realm of the formerly largest DNA viruses infecting the green algae *Chlorella*. A distant relative of the mimivirus was also found to infect the marine heterotrophic flagellate *Cafeteria roenbergensis*. Other mimiviruses have been reported to infect multicellular organisms, such as sturgeon, although none have been successfully cultivated. As new isolates are accumulating, it now appears that there is a continuum between the largest and regular-size viruses. Thus, giant viruses are not freaks of nature — they were simply overlooked due to established inappropriate filtering practices.

How do they replicate? Giant viruses infecting *Acanthamoeba* enter the cell by phagocytosis, mimicking microbial prey, hence the name 'mimivirus'. After capsid opening, the membrane lining the interior of the virus particle fuses with the phagosome membrane, delivering the virus DNA and associated proteins into the cytoplasm. Depending on the type of virus, their replication then follows very different paths, involving various cellular functions. The mimiviruses and pithoviruses rely on their own transcription machinery downloaded from the capsids together with their genomes. Thus, they do not need to enter the nucleus and can remain in the cytoplasm where they develop large viral factories. In contrast, no transcription machinery is present in the virions of the pandoraviruses and molliviruses. They thus have to transfer their genomes into the cell nucleus to gain access to the functions required for initiating their replication cycle. Interestingly, the marseilleviruses behave like an intermediate between entirely cytoplasmic and nuclear viruses. Despite the lack of particle-loaded transcription machinery, they end up installing their viral factories in the cytoplasm after temporally recruiting the required functions to the cytoplasm without transferring their genome into the nucleus. The replication cycles of all the above viruses is lytic, terminating in the assembly of mature virions that are then released by exocytosis or cell lysis.

Can giant viruses get infected? Yes, they can! Giant viruses with a cytoplasmic replication-cycle, such as mimivirus, can be targeted by other viruses, called 'virophages', which use their host's viral factory to replicate. Virophages are small DNA viruses with genomes of ~20 kb encoding about 20 proteins. They express their genomes as late genes using the giant virus transcription machinery. They either multiply alongside the giant virus without apparently affecting its replication (commensal) or impair it strongly enough (true parasite) to have a protective effect on the host cell population. Other companions of mimiviruses are called 'transpovirons'. Those are small double-stranded DNA molecules, about 7 kb long, that are replicated by the giant virus and propagated as episomes within its particles or within virophage capsids.

Did they change our view of the role of viruses in the environment? The discovery of the various families of giant and large viruses infecting *Acanthamoeba* has triggered a wider interest for viruses infecting unicellular eukaryotes. This has turned into a whole new research field in environmental virology. The sequence of the prototype genomes allowed the analysis of a huge body of metagenomics data and revealed the unexpected abundance and ubiquity of giant viruses in most aquatic and terrestrial ecosystems. Distant mimivirus relatives infecting various microalgae are now considered main players in the regulation of planktonic populations, as well as in oceanic elemental cycles by nutrient recycling (the viral shunt) and the reshaping of their host's metabolic network during infection. This contribution is probably underestimated as the large proportion of genes without previously known homologs in metagenomics datasets

might still correspond to virus families without characterized isolates.

What do we know about the origin and evolution of giant viruses? Despite their unexpected complexity, all giant viruses still obey Lwoff's most basic criteria distinguishing viruses from cells: they do not have an energy metabolism, they cannot synthesize proteins — there are no virally encoded ribosomes known yet, even if some mysteriously encode a complete set of amino-acyl tRNA synthetases and other translation-related functions. They remain obligatory intracellular parasites and do not multiply by binary fission. The discovery of multiple families of giant viruses sharing only a handful of core genes together with a large proportion of genes without cellular homologs raises the fundamental question of their origin (and that of viruses in general). Various origin scenarios are hotly debated, either postulating a single common ancestor or multiple independent origins. A consensus is nevertheless emerging that these ancestors probably predated the radiation of the eukaryotes, or even of all cellular life, making viruses members of a fourth domain of the tree of life. While we have proposed that giant viruses could have originated from various pre-cellular lineages and evolved by genome reduction, opposite scenarios see giant DNA viruses evolving by extensive gene acquisition from a transposon-like ancestor. Intermediate models see them evolving through alternating periods of genome inflation and reduction. Yet, none of these hypotheses address two fundamental questions: first, what kind of evolutionary process can generate intracellular parasites spanning the huge range of particle size and gene content exhibited by giant viruses? Second, what is the origin of the large proportion of proteins without homologues encoded in all giant virus genomes? An answer to the latter question might be provided by the recent finding that pandoraviruses and molliviruses are able to create proteins *de novo* from intergenic regions. Finally, in the absence of a common reference gene set for all DNA (or RNA) viruses, the only universal definition of viruses, as large as they could become, cannot be based on their immensely variable gene content, but on the unique way they propagate their genomes within metabolically inert particles.

Where can I find out more?

Abergel, C., Legendre, M., and Claverie, J.M. (2015). The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. FEMS Microbiol. Rev. *39*, 779–796.

Bäckström, D., Yutin, N., Jørgensen, S.L., Dharamshi, J., Homa, F., Zaremba-Niedwiedzka, K., Spang, A., Wolf, Y.I., Koonin, E.V., and Ettema, T.J.G. (2019). Virus genomes from deep sea sediments expand the ocean megavirome and support Independent origins of viral gigantism. mBio. *10*, e02497–18.

Christo-Foroux, E., Alempic, J.-M., Lartigue, A., Santini, S., Labadie, K., Legendre, M., Abergel, C., and Claverie, J.-M. (2020). Characterization of *Mollivirus kamchatka*, the first modern representative of the proposed *Molliviridae* family of giant viruses. J. Virol. *94*, e01997-19.

Claverie, J.M., and Abergel, C. (2018). Mimiviridae: An expanding family of highly diverse large dsDNA viruses infecting a wide phylogenetic range of aquatic eukaryotes. Viruses *10*, pii: E506.

Colson, P., Levasseur, A., Scola, B.L., Sharma, V., Nasir, A., Pontarotti, P., Caetano-Anollés, G., and Raoult, D. (2018). Ancestrality and mosaicism of giant viruses supporting the definition of the fourth TRUC of microbes. Front Microbiol. *9*, 2668.

Desnues, C., La Scola, B., Yutin N, Fournous, G., Robert, C., Azza, S., Jardot, P., Monteil, S., Campocasso, A., Koonin, E.V. *et al*. (2012) Provirophages and transpovirons as the diverse mobilome of giant viruses. Proc. Natl. Acad. Sci. USA *109*, 18078-18083.

Filée, J. (2015). Genomic comparison of closely related giant viruses supports an accordion-like model of evolution. Front Microbiol. *6*, 593.

Fischer, M.G., and Hackl, T. (2016). Host genome integration and giant virus-induced reactivation of the virophage mavirus. Nature *540*, 288–291.

Guglielmini, J., Woo, A.C., Krupovic, M., Forterre, P., and Gaia, M. (2019). Diversification of giant and large eukaryotic dsDNA viruses predated the origin of modern eukaryotes. Proc. Natl. Acad. Sci. USA *116*, 19585–19592.

Krupovic, M., Dolja, V.V., and Koonin, E.V. (2019). Origin of viruses: primordial replicators recruiting capsids from hosts. Nat. Rev. Microbiol. *17*, 449–458.

Legendre, M., Fabre, E., Poirot, O., Jeudy, S., Lartigue, A., Alempic, J.-M., Beucher, L., Philippe, N., Bertaux, L., Christo-Foroux, E., *et al*. (2018). Diversity and evolution of the emerging Pandoraviridae family. Nat. Commun. *9*, 2285.

Oliveira, G.P., Lima, M.T., Arantes, T.S., Assis, F.L., Rodrigues, R.A., da Fonseca, F.G., Bonjardim, C.A., Kroon, E.G., Colson , P., Scola, B.L. *et al*. (2017). The investigation of promoter sequences of Marseilleviruses highlights a remarkable abundance of the AAATATTT motif in intergenic regions. J. Virol. *91*, e01088–17.

Aix–Marseille University, Centre National de la Recherche Scientifique, Information Génomique & Structurale, Unité Mixte de Recherche 7256, Institut de Microbiologie de la Méditerranée, FR3479, 13288 Marseille Cedex 9, France.
*E-mail: Chantal.Abergel@igs.cnrs-mrs.fr

Quick guide

Zooxanthellae

Todd C. LaJeunesse

What are zooxanthellae? Until recently, all unicellular microalgae of yellow or brownish color found in animals or protists were customarily referred to as 'zooxanthellae', an antiquated term coined in the late 1800s. Animals that depend on photo-symbionts for their well-being were said to be zooxanthellate. In recent decades, advances in light and electron microscopy, combined with emerging molecular–genetic evidence, led to the realization that these photosynthetic symbionts represented many unrelated phyla of microeukaryotes. However, the large majority of 'zooxanthellae' are mutualistic dinofl agellates in the order Dinophyceae found in many shallow-water invertebrates (notably reef-forming corals), and in a few kinds of unicellular forams and ciliates. Dinofl agellates are a large and diverse group noted for their importance in plankton communities, as agents of harmful algal blooms (e.g., red tides) and in creating bioluminescence in the ocean. They share a recent common ancestor with the largely endoparasitic phylum Apicomplexa, some of which cause diseases such as malaria and toxoplasmosis.

In the second half of the 20th Century most dinoflagellatezooxanthellaewere formally classifi ed in the genus *Symbiodinium*; and originally thought to comprise one widespread species, *Symbiodinium microadriaticum*. However, the substantial genetic divergence between phylogenetic clades and large differences in their genomic compositions led to recent reorganization into multiple genera (currently nine) within the family Symbiodiniaceae, while other dinofl agellate orders and families contain symbiotic species, the family Symbiodiniaceae (order Suessiales) is by far the most geographically widespread and ecologically important.

Why are symbiodiniacean zooxanthellae important? Reef-building corals are ultimately reliant on the sun's radiance for their survival