Review Paper:

Virophage: The hijacker of my hijacker is my friend

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Abstract

The discovery of virophage carries along the proof of existence of a new bio controlling agent in the entire biosystem. The virophage is a parasite to a giant virus and works by hijacking "the giant virus" viral factory, an essential machinery for the giant virus's replication, leading to a sharp incline of the virophage viral load inside the host cell. Success of the host cell survival against the invading giant virus is shown by the decline of the destroyed cell during lytic stage after virophage co-infection to the giant virus. Virophage has a similar role to the bacteriophage but instead of targeting a bacterium, it targets specifically on virus. Hitherto, the existence of human-borne virophage and interactions of virophage to human microbiome remain elusive, thus future studies are required.

This short review will highlight the discovery, types and recent known method of virophage replication. We also added some biological perspectives of the connections and interactions between the virophage and its host to exploit the virophage main role as a biocontrolling agent to pathogenic viruses that are potentially benevolent for human life.

Keywords: Biocontrolling Agent, Giant Virus, Viral Factory, Virophage.

Introduction

Virus is the most abundant biological entity on Earth. Billions in number, virus has diversities on its genome types, size, virion shapes and replication cycles. They infect cells as reproductory mechanism ranging from bacteria, archaea, protozoa and other eukaryotes and each of virus type possesses unique and specific mechanisms[4,3]. Sometimes a specific replication mechanism of a certain group of virus on its host requires another virus as a proxy. Discovered in 1961, this group of virus is known as the satellite viruses[19,38].

Later in 2003, a group of massive-sized viruses, called the giant viruses (GVs) was discovered and they exhibit unique features.[2,7,34] Especially these GVs are known to possess greater complexity on their structures and genomes[37]. The viral replication occurs in the cytoplasm of the GV's host cell and a viral factory system is formed to assemble the GVs[17]. Compared to a small bacterium, a GV virion is much larger[30]. *Acanthamoeba polyphaga mimivirus* (APMV) is the first discovered GV with 500 nm in size with fibril length of 140 nm and houses a double-stranded DNA capable of encoding 979 putative protein[1].

A unique viral entity that resembles the satellite virus was discovered in 2008 and this virus possesses a paired-entry mechanism along with the GV during the intrusion to the host cell. This "satellite-like" virus hijacks the viral factory of the GV. For its capability to hijack a virus production to build its own viral structure, this entity is later called virophage[25]. Virophage inhibits viral replication of the GV inside the GV host cell, thus regulating the GV production in negative feedback control while this condition does not occur in normal satellite virus replication.[11,28] As it intervenes the GV viral factory reducing the chance of host cell to lysis, the virophage ensures the host cell survival[13].

To shed some lights for future applications of this virus, this review will highlight recent information of discovery, evolution, types and virophage replications. We also suggest some potential biological perspectives regarding the aspects of connections between the virophage and the viral host cell. From there, we might be able to reveal the virophage capability as a biocontrolling agent and its possible benefits to the human life.

What is Giant Virus?

Acanthamoeba polyphaga mimivirus (APMV) is the first GV to be identified in 2003 from an amoeba (specifically *Acanthamoeba*) culture and flow cytometry method under fluorescence staining.[2,7] Later, variants of *Mimivirus* have been identified from multiple ecosystems[34]. The DNA polymerase B family with a conserved region in an amoeba-infecting Mimivirus was discovered through a phylogenomic analysis and was predicted to possess three progeny types: A, B and C progeny types. Moumouvirus and Megavirus (*Megavirus chiliensis*) belong to type B dan C progenies, while APMV belongs to the type A[41]. The discovery of other GVs occured in 2010 and they were known to have distant relationship with the Mimivirus. They are *Cafeteria roenbergensis* virus (CroV) and this GV was discovered during infection of *C. roenbergensis*, a phagotrophic biflagellate organism[17]. Other GV, *Phaeocystis globosa* virus (PgV), was discovered when it infected a marine protest.[9,15]

A new family of GV was also recorded as *Marseilleviridae* in 2010[23]. However, the classification of other GV types is not yet accepted by the International Committee of Taxonomy of Viruses (ICTV) which include *Orpheovirus*, *Pandoravirus*, *Cedratvirus*, *Faustovirus*, *Mollivirus*,

Pacmanvirus, *Kaumoebavirus* and *Pithovirus*. These new viruses have a relation with the nucleo-cytoplasmic large DNA viruses (NCLDV), which previously were considered as monophyletic virus group that infects animal and unicellular organisms[32]. The NCLDV could trigger a formation of viral factories inside the host cell that serves as GV replication machine and later, morphogenesis[22].

The Sputnik Virophage

The new viral agent was discovered when the *Mimivirus* infected its host cell. This viral agent's replication process relies on a GV, *Mimivirus*[42]. This viral agent was later coined as a virophage, as it is morphologically similar to the bacteriophage. The discovery of this new virophage, named 'Sputnik', was made in 2008.[25,35] This virophage requires *Mimivirus* viral factory to produce its own 50 nm virion structure, which interferes with the GV replication infectivity and reduced the amoeba lysis rates.[12,36] Sputnik possesses double-stranded DNA as its genetic material with genome size of 18.343 pb consisting of major capsid (MCP), minor capsid protein (mCP) encoding genes and a suggested protein that involves in the DNA replication.

The Sputnik MCP sequence has 595 peptides, closely similar to *Mimivirus* MCP (473 peptides)[27]. However, no homology was detected between the Sputnik MCP and the *Mimivirus* MCP, thus Sputnik is suggested to evolve from other genetic elements before its association with the *Mimivirus*[28].

The Maverick-like virus: *Mavirus* Virophage

An aquatic *Mavirus* virophage performs co-infection with a CroV, a GV distantly related to the *Mimivirus*[14]. The host of this GV is an aquatic flagellate, *C. roenbergensis*. The similar genomic comparison with the *Mimivirus* family allows CroV to be included into the virus family members[5]. The Sputnik and the *Mavirus* are quite similar in term of capsid and genomic size (60 nm and 19.063 pb for both). The *Mavirus* inhibits the CroV replication, increasing the host cell survival rates[27]. Previous study on the *Mavirus* genomic identification revealed the evolutionary relationship between the virophage and the Polintons (also known as Mavericks) transposable element.

Fascinatingly, both the Polintons and the *Mavirus* possess conserved regions in the protein-primed DNA polymerase B (PolB) genes and the rve-superfamily retroviral integrase (rve-INT) genes. Hence, the Polintons can be referred as the evolutionary result from virophage ancestor, based on the size and viral morphologic similarities between the polintons and the *Mavirus*[16].

The Zamilion Virophage

A virophage was discovered in Tunisia in 2014, called 'Zamilion'. The difference between the Zamilion virophage and the Sputnik is that the Zamilion virophage does not reduce GV infectivity and replication[18]. The Zamilion is a unique virophage for its capability to replicate from *Mimivirus* viral factory and lineage, which is not the A type, but instead the B and the C types[6]. The GV host of the Zamilion virophage is identifiable as it produces a "molecular weapon" of the virus called mimivirus virophage resistance element (MIMIVIRE) and this serves as the GV defense mechanism[10]. The Zamilion virophage has approximately 17.276 pb of double-stranded DNA with 20 genes. The similarities between the Zamilion and Sputnik genes are identified at 31-86%[27]. However, it is not yet identified whether the Zamilion virophage will evolve to counter the MIMIVIRE in similar manner to the bacteriophage virus against bacterial CRISPR-Cas defense system.

Other Virophages

Other virophages were discovered from a metagenomic dataset, a culture isolation and a co-culture experiment. A virophage that resembles Sputnik, 'Guarani' was discovered in 2019 in the Pampulha Lagoon, Belo Horizonte, Brazil. The coexistence of Guarani with the GV natural host can be found in the nature. The Guarani virophage has a double-stranded DNA genomic type with 18.967 pb and encodes 22 proteins which are similar to the Sputnik[27].

The other virophage, Sissivirophage, was discovered but has not been fully characterized and is metagenomically homologous with the data from a virophage found in Lake Mendota, USA[32]. Another virophage was found when a genomic sequencing was performed on phycodnavirus (PgV-16T) and it is called phaeocystis globosa virus virophage (PgVV). The PgVV has double-stranded DNA genomic type that is capable of encoding 16 proteins[36].

Virophage Evolution Puzzle

Transposable elements like Polintons are contained in various eukaryotic genomes[37]. Two kind of enzymes encoded by all Polintons are an integrase and protein-primed type B DNA polymerase.[20,37]. Some Polintons encode ATPase, two capsid proteins (double jelly-roll fold), a mCP, a C5-family protease and a MCP. The morphogenesis genes of the virus provide some evidences that under a certain condition, the mobile genetic element allows the creation of the virion (polintonviruses), the gene takes two important roles on the viral assembly and the transposable element[43].

This condition rises further questions about the evolutionary connections between the virophage, the GV of ordo *Megavirales* DNA, the bacteriophages and the Polintons. However, the polinton virus was suggested as the first viral entity resulting from bacteriophages evolutionary line and became the ancestor of the most eukaryote DNA viruses like the GVs and the virophages.[24,43]

About 3 virophages, the Organic Lake virophage, the Sputnik and the *Mavirus* were phylogenomically analyzed and revealed six shared proteins that are homologous in all tested virus, those proteins are cystein protease, Zn-ribbon domain containing protein, mCP, packaging ATPase, Primase Superfamily 3 helicase and MCP. Four core genes

of the virophage including the maturation protease and packaging ATPase are also available in the Polintons[43].

However, two virion proteins, the MCP and the mCP are not homologous outside the virophage group which reveals an idea that the virophages are evolved from a single ancestor. Some older studies indicate that the virophages evolved through a joined recombination of the Polintons and then the ancestor of the virophage could co-infect through the GV infected cells. Another scenario suggested that the Polintons was derived from a virus and the virophage originated from the Polintons evolutionary line but from the unknown shared ancestor[8].

Virophage Replication

Entry: The virophage undergoes viral entries based on two different models (Figure 1). The Sputnik virophage is known to use the paired-entry mode where they are paired with the host virus to enter the host cell through the GV's fibril attachments[6]. The virophage capsid part then interacts with the GV's peptidoglycan-like structure allowing the entrance into the host cell through paired-entry mode to happen.[27,28] The second entry model found in the *Mavirus* is via clathrin-mediated endocytosis which does not rely on the GV to enter[21]. Despite these mechanisms already being identified and described, further studies are ongoing to reinforce the details.

Eclipse Phase: In the amoeba cytoplasm, there are endocytic vacuoles normally formed about 1-2 hours after infection. The eclipse phase occurs next within 2-4 hours after infection followed by the viral genomic replication, transcription and translation. The GV (*Mimivirus*) and the virophage (Sputnik) were characterized to perform genomic replication in the host cell cytoplasm and the viral factory structures were also discovered in form of small spheres. It is unlikely to isolate the virophage viral particles during this phase.

Spreading: The viral factory then expands after the completion of the eclipse phase and at that moment, the complete virions of the virophage is produced[17]. At alternative conditions, the viral factory only produces the *Mimivirus* on the part if the virophage interference is absent. After 16 hours, the amoeba cytoplasm will be saturated by the new virophage and Mimivirus virion particles. The accumulation of the virophage particles will be detected on the amoeba host cell vacuole and cytoplasm. After 24 hours, the amoeba host cell will rupture (lysis) under normal non-virophage interference condition and the viral loads of the *Mimivirus* will be higher than the virophage[29]. As virophage is much smaller, their release will not damage the amoeba cell as the GV.

Cell Host Survival

Most of the virophages negatively affect the replication of the host GV significantly. The *Mavirus* and the Sputnik virophages decrease the value of the GV host viral loads while increasing the host cell survivability.[26,27] Until now, only few virophages have been obtained from the GV that infects *Sccamoeba* spp. These virophages drop the replication rates of the GV at the capsid formation stage up to 70%, which helps increasing the amoeba survival rates when infected by the GV[26]. The interactions between the virophage, the GV and the host cell can be observed instrumentally (Figure 2).

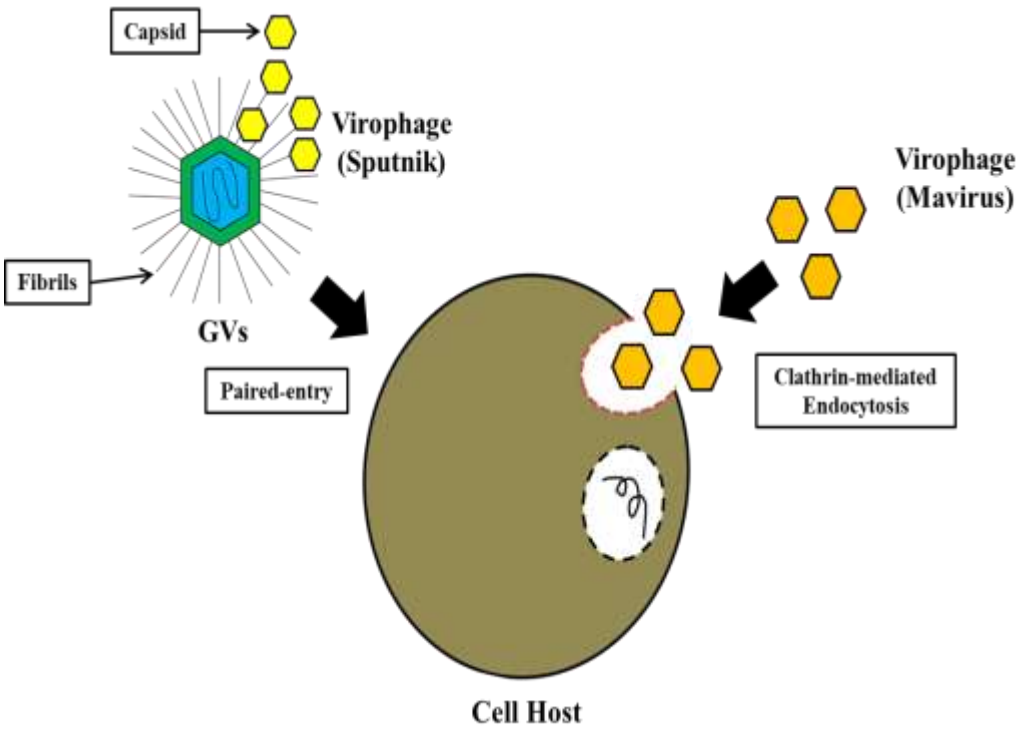

Figure 1: The virophage replication models

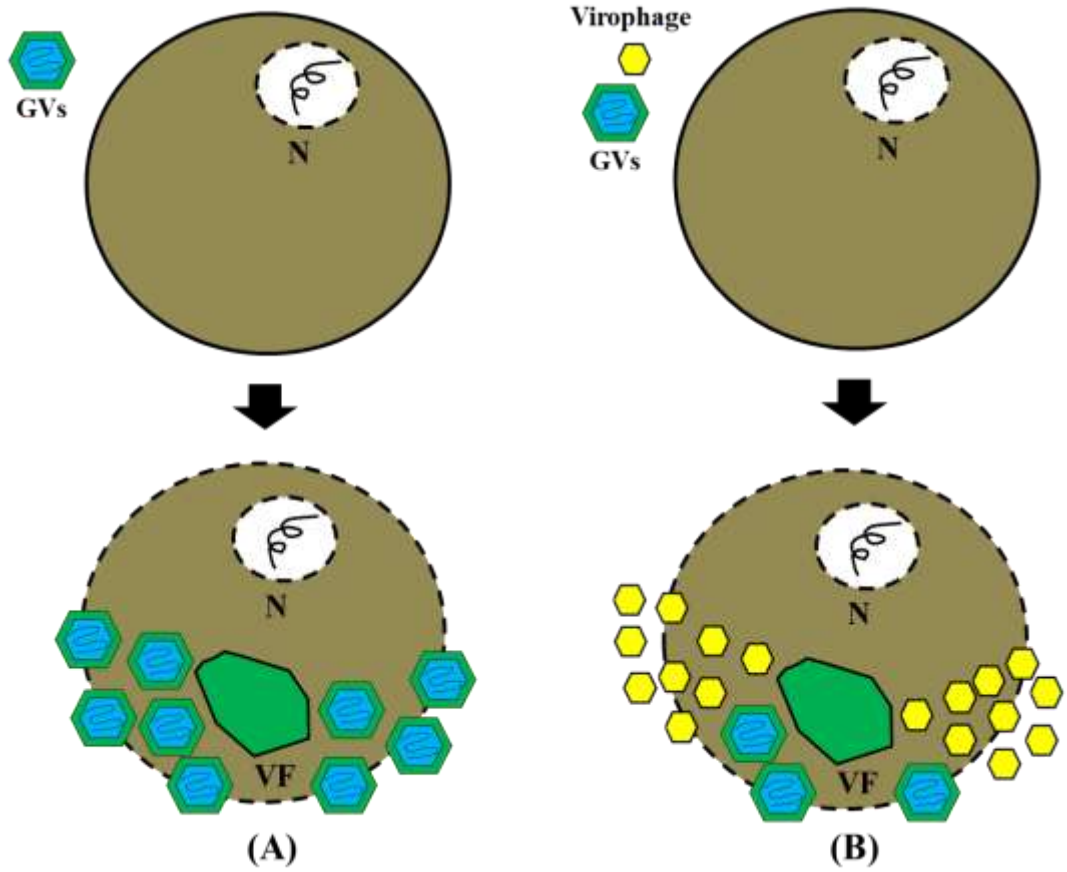

Figure 2: The interactions between the virophage, the GVs and the host cell. N: Nucleus, VF: viral factory

The virophage hence contributes in a regulation process of the unicellular organisms population dynamics, especially for the marine protists including amoeba. A stimulation of growth rate, survivability and drop on the mortality rates was observed in the population of photrophic algae during a virophage sampling in the Organic Lake, Antartica[40].

Biological Perspectives

The discovery of virophage leads us to a new proof of a new biocontrolling agent in the biosystem on Earth. The virophage serves as a regulator of the GV by hijacking its viral factory for its own replication, allowing the host cell of GV to survive dramatically, preventing the host cell from lysis as the virophage co-infects the cell along with the GV. The relationship between the virophage and the GV-infecting an amoeba is symbiosis parasitism.

We generally know that a virus is a pathogen which is always considered malevolent as it infects the host cell. Now, as we have knowledge about the virophage, we finally know that there is a special virus that parasitizes another virus, hence keeping the host cell alive. Similar to the bacteriophage in terms of a controlling agent, the virophage host is a GV while bacteriophage's host is a bacterium (Figure 3).

The virophage might be potentially useful to control human or animal pathogenic viruses in the future. More studies of the virophage that involve co-culturing with animal or human cells and pathogenic viruses are required to unlock its potential and to understand its interactions with cells other than just amoeba.

Perhaps there are more virophages out there and some could be inside an animal or a human body as part of our microbiome. Now, we can understand how viruses can potentially be beneficial, instead merely just a hijacking pathogenic machinery. As it also serves as a biocontrolling agent against another viruses, perhaps in the future we can say to the virophage, "the hijacker of our hijacker is our friend".

Conclusion

Virophage is an extraordinary entity in the realm of virus. Although most details about virophage are currently elusive, more studies are required in the future to understand it better. The virophage is a biocontrolling agent that regulates the giant virus (GV) population as it parasitizes the host cell (amoeba), thus alllowing the host amoeba cells to survive.

The virophage holds a similar role as the bacteriophage, despite the target is viruses (the GV) instead of bacteria. The interaction of the virophage with human body and microbiomes and whether we harbor our own virophage is yet unknown. Therefore, future studies are greatly encouraged.

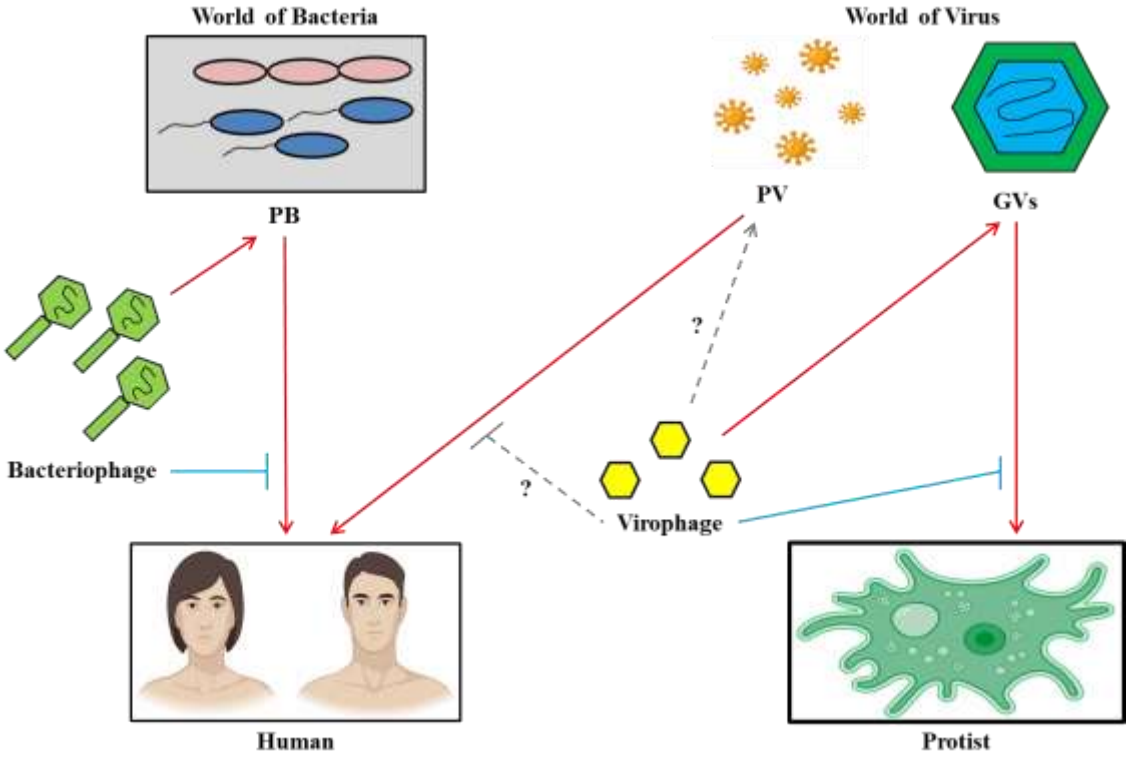

Figure 3: The mode action comparison between the bacteriophage and the virophage in the biosystem. PB: Pathogenic bacteria, PV: Pathogenic virus

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