REVIEW

The parasite's parasite: Exploring virophages' antiviral strategies in developing current and novel antiviral therapies

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ABSTRACT

Pathogenic viruses exert a profound impact on a population by infecting and parasitizing their hosts. Apart from the immune system, there are few known natural mechanisms that inhibit the replication and propagation of viruses within a cell. Recently, a new class of subviral particles, virophages, has been identified and described. They parasitize giant viruses and limit their growth within a cellular population. In this review, we explore the parasitic and antiviral tactics employed by sputnik and mavirus, the two most well-characterized virophages, against their viral hosts. Additionally, we discuss how their strategies compare to existing antiviral therapies and highlight specific areas of research that may help fully elucidate the virophages' mechanisms, which offer promising insights into the development of existing and novel antiviral therapeutics.

INTRODUCTION

Exploration into the diversity of life has elucidated a multitude of interspecies relationships and interactions. In microbiology, understanding the interactions between microorganisms has enabled the development of invaluable tools and strategies that proved useful in the fields of medicine and research (Hitchcock et al. 2023; Fruciano and Bourne 2007).

Viruses stand as the most diverse and ubiquitous infectious agents known today. As obligate intracellular parasites, their success relies on their ability to hijack cellular hosts and their protein machinery (Flint et al. 2020; Gelderblom 1996). With such strategy, many groups of viruses have been identified to pose significant threats to the health of various organisms, including humans, as demonstrated by recent events with the COVID-19 pandemic (Kausar et al. 2021). While vaccines have shown significant promise and efficacy in disease prevention and severity reduction (Moghadas et al. 2021), the need for effective and safe antiviral treatments remains imperative for managing post-infection cases, limiting viral pathogenicity, and treating immunocompromised individuals. Unfortunately, the development of antiviral strategies comes with significant obstacles. Viruses' ability to mutate rapidly and adapt to existing treatments poses a constant challenge (Irwin et al. 2016). Additionally, the intricate interplay between a virus and its cellular host makes it difficult to create drugs that selectively target the virus without harming the host cell. Therefore, identification of novel sources for antiviral strategies and agents is an important emerging field for health research.

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In 2008, a novel subviral particle named sputnik was discovered to parasitize the giant viruses *Acanthamoeba polyphaga mimivirus* (APMV) and *Acanthamoeba castellanii mamavirus* (ACMV), and promote the survival of the cellular hosts, *A. polyphaga* or *A. castelanii*, respectively (La Scola et al. 2008). Shortly thereafter, mavirus was identified and characterized, specifically observed to parasitize *Cafeteria roenbergensis* virus (CroV), which infects the marine flagellate, *C. roenbergensis* (Fischer and Suttle 2011). Unlike other subviral particles, sputnik and mavirus' genomes are generally larger in size; they independently encode for various virophage genes, including capsid proteins for particle assembly, an ATPase for genome packaging, and an integrase that enables insertion of their genomes within the hosts' (La Scola et al. 2008; Fischer and Hackl 2016; Fischer 2012). Additionally, they specifically replicate in the presence of their giant virus hosts, hijacking their viral machinery within the viroplasm (Fischer and Suttle 2011; La Scola et al. 2008). Since then, the discovery of other subviral particles categorized with similar properties and genomic organization have been described (a recent review of this topic is covered in Tokarz-Deptula et al. 2024). Collectively, they were termed 'virophages', grouped in the *Lavidaviridae* family as *bona fide* intracellular parasites of giant viruses and were theorized to have originated as transposable elements from their cellular hosts (Tokarz-Deptula et al. 2024; Koonin and Krupovic 2017; Fischer and Suttle 2011; La Scola et al. 2008).

Majority of articles on virophages focus on isolation, identification, and characterization of novel strains (Tokarz-Deptula et al. 2024; Fischer and Suttle 2011; La Scola et al. 2008); very few delved on their unique antiviral strategies and comprehensively discussed their potential for antiviral therapeutics. This narrative review explores the intricate interplay between virophages, viruses, and their cellular hosts (Figure 1), offering invaluable insights that may be utilized for the development of antiviral therapy.

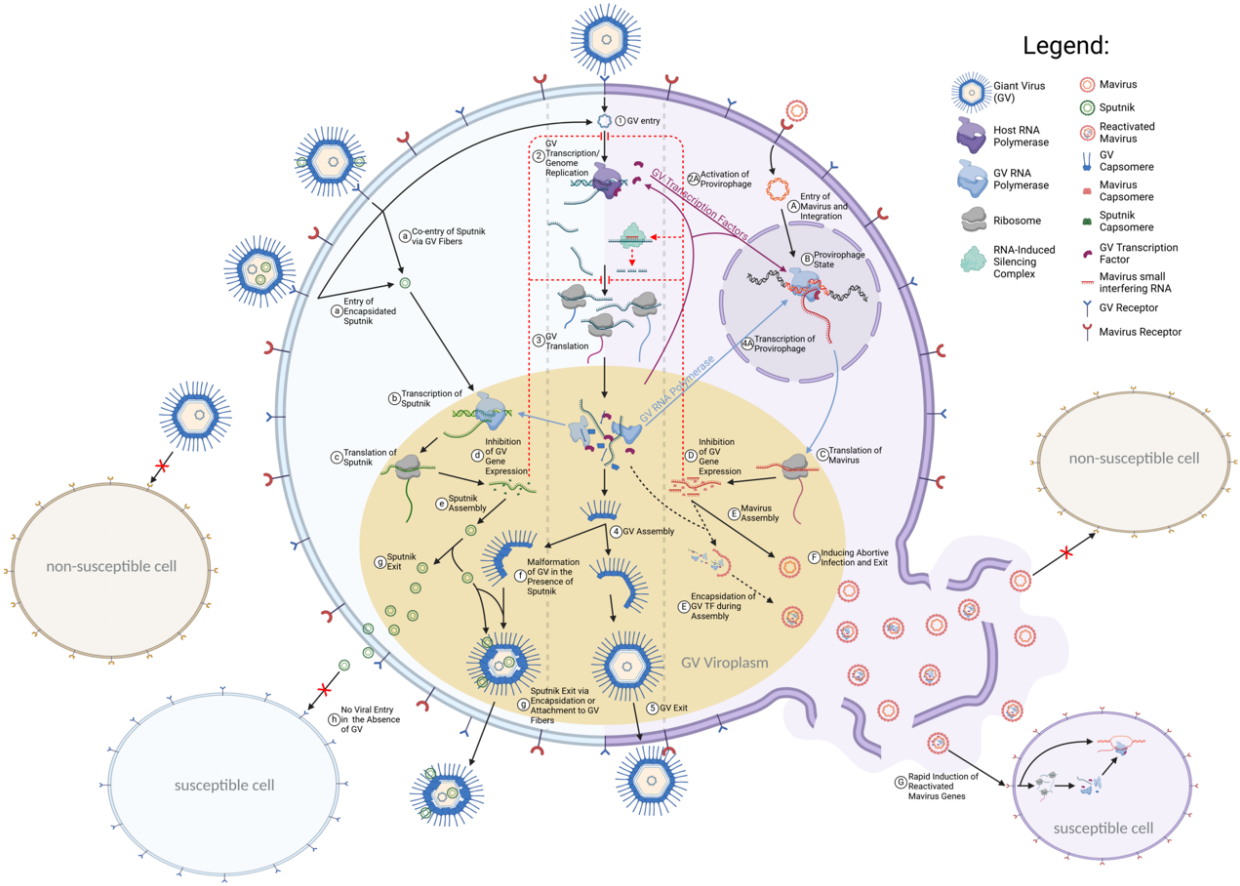

Figure 1: Replication cycles of sputnik (left; a-h), giant virus (middle; 1-5), and mavirus (right; A-G). Sputnik specifically targets virally susceptible cells using giant virus as a vector (a), it replicates in the viroplasm (b-g) while inhibiting viral gene expression (d). Giant virus enters receptor-expressing (susceptible) host cells (1). It hijacks host cell machinery to replicate viral genome and proteins (2-3); it expresses its genes and assembles in the viroplasm (3-5). Mavirus independently enters a cellular host (A), and enters a provirophage state in the absence of giant virus (B). Upon viral infection, mavirus is activated by the expression of viral transcription factors (4A; C-F), and inhibits gene expression of giant virus (D). Reactivated mavirus is packaged with necessary viral proteins to efficiently inhibit viral propagation during subsequent infections (G).

To provide a representative overview of virophages' parasitic properties against their viral hosts, we delve into the antiviral strategies employed by sputnik and mavirus, the two most well-characterized virophages (Tokarz-Deptuła et al. 2023). These virophages, representing the two distinct genera of *Lavidaviridae* (*Sputnikvirus* and *Mavirus*) (Paez-Espino et al. 2019), have revealed insights into various parasitic tactics against viruses. Specifically, we examine sputnik's virus-dependent entry, sputnik and mavirus' reliance on viral components for replication, and mavirus' induction of abortive infection. By comparing these strategies to existing antiviral treatments, we aim to highlight the potential of virophages as a model of novel antiviral therapies and identify future research areas that could lead toward this direction. Overall, the mechanisms employed by these subviral particles offer promising avenues for the development of existing and innovative approaches to combat viral infections effectively.

A. PARASITIC STRATEGIES OF VIROPHAGES AGAINST GIANT VIRUSES

Virus-dependent entry of virophages promotes cellular host specificity

Virophages require host cells to persist, similar to viruses. One unique characteristic of virophages points to the possibility that they can independently enter a cellular host without their viral counterpart unlike many subviral particles (Tokarz-Deptula et al. 2023). Specifically, they are known to encode their own capsid protein subunits (La Scola et al. 2008; Tokarz-Deptula et al. 2023), likely containing the appropriate receptor and/or surface that can mediate host cell entry. As such, virophage entry can occur via host cell receptor-mediated endocytosis, similar to viral entry (Fischer and Suttle 2011). However, there is strong evidence that virophages, particularly sputnik, may also take on alternative virus-dependent routes of infection.

Boyer et al. (2011) showed that a bald strain of APMV (M4) lacking capsid fibers appears to be resilient from sputnik's parasitism. Notably, this presents the possibility that sputnik externally attaches to viral capsid fibers to co-enter cellular hosts along with its viral counterpart. A similar feature has been reported with a satellite called miniflayer. Satellites are another group of subviral agents dependent on their helper virus for co-infection of a cellular host (Fischer 2012; deCarvalho et al. 2023). In this case, miniflayer has been shown to latch onto the tail of P4 bacteriophage to help facilitate its entry into *Streptomyces* (deCarvalho et al. 2023).

Alternatively, transmission electron microscopy also revealed that a percentage of sputnik particles may become internally encapsidated within ACMV particles upon host cell exit (La Scola et al. 2008). Since sputnik encodes for its own major capsid (ORF20) and minor virion proteins (ORF 08, 19) (La Scola et al. 2008; Zhanga et al. 2012), it is interesting to observe the virophage's presence inside viral particles. Although it is not unlikely for the virophage to be packaged inside the larger viral virion, sputnik's encapsidation within a viral capsid may also adaptively facilitate its entry within its cellular host. Viral receptors present in the capsid allows cell-type specific entry of viruses. Hence, the utilization of viral capsids by virophages may strategically facilitate their entry within host cells, specifically susceptible to the same viral infection.

Sputnik and Mavirus temporally activate in the presence of their viral counterpart

Virophages are known to specifically activate their genome in the presence of viral infection. Arguably, this strategic mechanism of virophages results from their high genetic similarities with their viral hosts. To explain, giant viruses are known to encode many of the proteins necessary for their own gene expression (Mougari et al. 2019), including transcription factors and RNA-processing proteins. These regulatory proteins recognize and bind to conserved regions encrypted within the viral DNA or transcripts. As transposable elements, virophages have likely acquired these viral consensus sequences via horizontal gene transfer, which would enable virophages to hijack viral proteins specifically upon infection via viral-dependent regulation of their gene expression.

In the absence of its viral counterpart, mavirus enters a dormant provirophage state. Its encoded integrase facilitates the insertion of its genome within *C. roenbergensis* (Fischer and Suttle 2011), where the virophage's genes become minimally expressed (Fischer and Hackl 2016). However, in the presence of CroV infection, maviral gene transcripts begin to increase significantly (Fischer and Hackl 2016; Koslova et al. 2024). This elevated expression has been shown to depend on CroV protein translation, specifically the syntheses of its late transcription factors. These regulator proteins enhance gene expression by binding to their corresponding consensus promoter sequences, identified to also be present within the maviral genome (Fischer and Hackl 2016). Consequently, increased expression of maviral genes by CroV transcription factors would allow the virophage to transition from a quiescent to a replicative state. In fact, the observed mavirus genes with elevated expression include: MV03 (DNA polymerase), MV15 (genome packaging ATPase), MV16 (maturation protease), MV17 and MV18 (minor and major capsid proteins, respectively) (Fischer and Hackl 2016), proteins necessary for production of new mavirus particles (Koonin and Krupovic 2017). This virus-dependent regulation of virophage genes presents a molecular mechanism through which virophages ensue a transcriptionally active and replicative state specifically upon viral infection (Claverlie and Abergel 2009; Suhre et al. 2005).

Investigation of APMV and sputnik's RNA transcripts presents another potential strategy by which virophages temporally activates gene expression during viral infection. In the nucleus, transcribed RNAs are critically capped and processed at their 5' and 3' ends with a 7-methylguanosine and a poly-A tail, respectively (Flint et al. 2020). Since giant viruses remain in the cytoplasm, they have developed strategies that allow RNA processing, independent from their cellular hosts. They encode genetic consensus sequences or structures recognized by their own unique viral proteins that would facilitate either 5' capping or 3' poly-A tail synthesis (Flint et al. 2020; Claverlie and Abergel 2009). For example, APMV is observed to have a hairpin structure at the 3' ends of its m- and t- RNA transcripts; it has been suggested that this structure is recognized and polyadenylated by its own unique viral proteins R343 and R341 (poly-A polymerase subunits), respectively (Byrne et al. 2009). Interestingly, the hypothesized 3' hairpin binding site of these proteins is also found in various sputnik's transcripts, but not in any of its cellular host's RNAs (Claverlie and Abergel 2009; Byrne et al., 2009). This suggests that sputnik specifically relies on APMV's R341 and R343 proteins to acquire the 3' poly-A tails for its transcripts. Given the importance of this mRNA feature for stability and efficient translation (Flint et al. 2020), it can be deduced that the temporal activation/expression of sputnik genes specifically during virus infection, is the result of their critical dependence on APMV proteins.

The replication cycles of giant viruses are known to be entirely cytoplasmic (Flint et al. 2020). As such, it remains a mystery how pro/virophages acquire the necessary viral proteins for transcription and/or transcript processing, if their genomes are present in the nucleus. For provirophages that become integrated in the host's genome, such as mavirus, it has been hypothesized that its required viral proteins contain a nuclear localization sequence (NLS) that can hijack cellular host's translocation system via nucleopores (Fischer and Hackl 2016; Liu et al. 2017). Another possibility is the excision of the provirophage from the cellular host's genome, likely triggered by detection of viral infection, followed by its transport to the viroplasm, where virophage replication is shown to be highly concentrated (Fischer and Hackl 2016; Claverlie and Abergel 2009). Alternatively, when a virophage co-enters with its virus, either attached to its capsid (Boyer et al. 2011) or genetically integrated in the viral genome (Koonin and Krupovic 2018; Tokarz-Deptula et al. 2024), it would remain and replicate in the cytoplasm where the requirement for an internuclear transport is no longer needed.

Sputnik dysregulates viral gene expression

APMV or ACMV infection of *A. polyphage* or *A. castelanii,* respectively, causes rapid intracellular viral replication, resulting in cell lysis to induce viral release (La Scola et al. 2008). However, in the presence of sputnik, the rate of viral gene

synthesis and virion assembly are significantly reduced by 70% as production of virophage particles becomes kinetically favored. Additionally, sputnik also induces malformation of viral capsids, effectively limiting overall viral propagation and promoting cell population survival (La Scola et al. 2008).

Within the cellular host, the replication of sputnik has been shown to occur in the viroplasm (La Scola et al. 2008). This presents the possibility of virophage interference against viral genome replication and/or gene expression, which also occur at this site. Viroplasms are proteinaceous viral factories found in the cytoplasm of infected cells where viral replication processes, including genome replication and protein synthesis, are found to be highly concentrated (Mougari et al. 2019). The presence and increased production of virophage in viroplasms, along with the reduced production of viral particles, serves as foundational evidence that sputnik hijacks the viral machinery present at this site to favor its own particle synthesis.

The exact mechanisms behind sputnik's parasitism of its viral host remain to be fully elucidated. However, their previously discussed genetic similarities present one possibility of how sputnik hijacks viral regulatory proteins to induce its own RNA processing. Additionally, it has also been observed that the majority of nucleotides constituting APMV, ACMV and sputnik's genomes are adenines (A) and thymines (T) (La Scola et al. 2008; Colson et al. 2013), unlike their guanine-cytosine rich cellular hosts (Colson et al. 2013). Coincidentally, APMV has been shown to independently encode for many of its tRNAs and its own aminoacyl-tRNA synthetases, to accommodate and enhance viral gene expression (Abergel et al. 2007). This allows the virus to rapidly synthesize a pool of amino acids within the viroplasm that likely corresponds to its A-T rich genome (Colson et al. 2013; Zhanga et al. 2012). However, given its similar genome composition and spatial production site as sputnik, it is plausible that the viral aminoacyl-tRNAs are hijacked and used for translation of virophage proteins. This would consequently drive and fuel protein translation of sputnik within the viroplasm (Zhanga et al. 2012; Colson et al. 2013), effectively promoting its replication while simultaneously limiting APMV particle production as translational 'resources' become depleted.

To support this further, abnormal APMV and ACMV particles with thicker capsids and asymmetric virions (La Scola et al. 2008) are also observed in the presence of sputnik. These abnormalities in the viral capsid morphology specifically implicate sputnik's role in dysregulating viral protein synthesis. Capsids are composed and assembled through quasi-equivalent interactions among their protein subunits (Gelderblom 1996; Flint et al. 2020). Therefore, obstruction of protein synthesis in any of these subunits during transcription or translation would lead to an overall defective capsid structure. Consequently, these abnormal structures would compromise their critical role in genome protection, viral exit and subsequent infections (La Scola et al. 2008; Mougari et al. 2019).

Mavirus induces abortive infection and prevent subsequent infections

Mavirus also replicates in the viroplasm of its viral host, CroV, while dysregulating viral gene expression (Fischer and Hackl 2016; Koslová et al. 2024), similar to sputnik. Interestingly, CroV-dependent expression of maviral genes is reported to sporadically result in cell death of the cellular host (Fischer and Hackl 2016; Koslová et al. 2024). Nonetheless, it effectively limits the increase of viral genome and improves the survival of the cell population as a whole (Fischer and Hackl 2016; Koslová et al. 2024). As such, it has been suggested that mavirus induces abortive infection, which selectively triggers cell death among CroV-infected cells, but strategically promotes the survival of uninfected ones (Fischer and Hackl 2016; Koslová et al. 2024).

Upon infection, mavirus becomes transcriptionally active and begins to parasitize CroV, while promoting production of its own virophage particles (Fischer and Suttle 2011). Interestingly, this first round of infection may result in abortive infection (Fischer and Hackl 2016), likely induced via DNA degradation, cellular metabolite depletion, and/or disruption of membrane integrity (Boyle and Hatoum-Aslan 2023). These changes would limit the production of viral particles, but also allow the release of newly synthesized virophage particles (Koslová et al. 2024). Notably, these second generation virophages released from the infected population, have been shown to significantly inhibit subsequent rounds of viral replication and propagation. As such, it has been hypothesized that the continued spread of the mavirus would result in complete viral clearing within the cellular population (Koslová et al. 2024).

The multi-faceted antiviral system of mavirus presents a significant finding. Besides parasitizing giant viruses in the primary cellular host (La Scola et al. 2008; Fischer and Hackl 2016), evidence suggests that mavirus further inhibits viral propagation during subsequent rounds of infection (Koslová et al. 2024). Interestingly, the secondary propagation of mavirus is believed to significantly enhance viral clearing within a population. Although the underlying mechanisms remain unknown, one hypothesis is that 'reactivated', second-generation virophage particles are packaged with viral proteins, important for gene expression (i.e., viral transcription factors). With this, infection of cells with these virophages would undergo a temporary antiviral state, where the maviral genes are automatically expressed upon cell entry, thereby decreasing viral susceptibility and/or permissiveness. However, since these viral regulatory proteins are not encoded in the virophage genome, their effects would be transient in the absence of viral infection. After complete viral clearance, the virophage then re-enters an inactive, provirophage state until another infection initiates its reactivation.

B. VIROPHAGE-MEDIATED VIRAL PARASITISM PRESENTS IDEAL STRATEGIES FOR ANTIVIRAL THERAPY DEVELOPMENT

Viruses are ubiquitous and capable of infecting all forms of life. Among these are disease-causing viruses that pose significant risks to the public health and human livelihoods (Victoriano-Belvis et al. 2021; Cheng et al. 2022; Wilkinson et al. 2011). Besides the recent COVID-19 pandemic, the Philippines is constantly threatened by various mosquito-borne viral infections (Kobayashi et al. 2017; Nabeshima et al. 2014), including dengue, to which there is currently no approved vaccine for prevention (Victoriano-Belvis et al. 2021; Ylade et al. 2024). Therefore, the development of antiviral treatments is important for various sectors of our society.

It is important to note that pathogenic viruses, though they may possess similar features, are still unique from the giant viruses described above. Nonetheless, the mechanisms employed by virophages present efficient antiviral techniques that substantially reduce production of their viral hosts. Here, we highlight how antiviral strategies employed by virophages display similar features as existing antiviral therapies (summarized in Table 1). Additionally, we discuss how elucidating the molecular mechanisms behind these strategies could reveal potential avenues for the development of both existing and new antiviral treatments.

Table 1: Similar Strategies Employed by Virophages and Existing Antiviral Therapies

Virophage Mechanism	Description	Examples of Existing Strategies
Attachment to viral fibers and/or encapsidation inside viral capsid	Virus-dependent entry of virophages through the utilization of viral capsid and/or fiber proteins (La Scola et al. 2008, Boyer et al. 2011, Dutta et al. 2021) allow cell-type specific entry, primarily targeting virus-susceptible and/or infected cells.	Viral vectors: Adeno-associated virus, rabies, etc. (mostly for research)
Temporal Activation	Sputnik and mavirus are dependent on their viral host. In the absence of infection, mavirus enters an inactive, provirophage state. The presence of viral proteins (transcription factors or amino-acyl synthetases) activate virophage's antiviral system (La Scola et al. 2008, Fisher and Hackl, 2016, Fischer, M. 2012).	Kinase-dependent nucleoside analogs
Inhibition of viral genome replication or gene expression	Sputnik and mavirus replicates in viroplasm. (La Scola et al. 2008, Fisher and Hackl, 2016, Fischer, M. 2012, Dutta et al. 2021) This feature allows virophages to hijack the viral machinery and employ kinetically advantageous antiviral mechanisms.	Integrase inhibitors, nucleoside analogs, reverse transcriptase inhibitors, polymerase inhibitors, miRNAs
Interference of viral assembly and exit	Sputnik prevents virion assembly by disrupting capsomer synthesis and/or hijacking of viral capsids (La Scola et al. 2008).	Viral protease inhibitors
Induction of abortive infection	Upon viral infection, mavirus triggers the cellular host's antiviral system, inducing abortive infection (Fisher and Hackl, 2016) . This prevents virus propagation by limiting the spread of infection.	Nucleoside analogs, interferons
Inhibition of viral propagation and induction of antiviral defense	The release of mavirus prevents further viral infection (Fisher and Hackl, 2016, Koslová et al. 2024). Increasing the multiplicity of infection of mavirus completely eliminates viral propagation within a population.	Interferons, antibodies (Passive immunization)

Selective targeting of cellular host

Evidence suggests that virophages employ molecular strategies that enable entry within virally-susceptible cells (Fischer and Hackl 2016; Boyer et al. 2011). Although they encode for their own protein coat (La Scola et al. 2008) and can independently enter a cellular host (Fischer and Suttle 2011), viral fiber-dependent entry or viral encapsidation present strategically relevant antiviral delivery systems that selectively target virus-susceptible cells. Therefore, identifying viral fiber attachment site/s and understanding how virophage hijack viral virions present future areas of investigation. Theoretically, these strategies would prevent antiviral absorption by cells that are naturally resistant to virus infection, minimizing the required amount to effectively inhibit viral replication within a heterogeneous cellular population.

In research, viral capsids can be utilized as vectors to facilitate the delivery of exogenous genetic material into specific tissues (Naso et al. 2017; Fischer et al. 1997). Viruses are known to be cell-type specific, their entry is mediated by interactions between host cell receptors and viral ligands found within the capsid or viral lipid membrane. This contributes to the virus' tropism and tissue- or cell-type specificity. For example, adeno-associated virus' projections bind to growth factor receptors and/or integrins present in fibroblasts and hepatocytes (Naso et al. 2017; Asokan et al. 2006); meanwhile, rabies virus glycoproteins bind to nicotinic acetylcholine receptors and neuronal cell adhesion molecules to infect nervous tissues (O'Brien et al. 2024; Lafon 2005). As such, the utilization of viral capsids as vectors to selectively target and express antiviral compounds within virus-susceptible or virus- infected cells may be considered as a potential avenue for an antiviral delivery system.

Viral protein specificity and temporal activation

Many intracellular antiviral compounds have been designed to target different aspects of viral replication. This includes nucleoside analogs that terminate the synthesis of genetic materials (Frobert et al. 2005); inhibitors of reverse transcriptase and integrase that prevent reverse transcription and viral DNA integration, respectively; microRNAs that inhibit viral translation; and viral protease inhibitors that suppress viral protein cleavage involved in viral release (i.e., influenza virus neuraminidase) (Kausar et al. 2021; Flint et al. 2020). Unfortunately, many of these antiviral treatments have the tendency to also suppress various cellular processes.

Virophages minimize their damage in a cellular population by employing a temporal- and protein-specific strategy (Fischer and Hackl 2016; Tokarz-Deptula et al. 2023). Both sputnik and mavirus do not replicate in the absence of their viral counterparts (La Scola et al. 2008; Fischer and Hackl 2016; Koslová et al. 2024). Therefore, it has been hypothesized that virophages are dependent on specific viral proteins, which allow temporal activation. Specifically, mavirus binds viral transcription factors to enhance its genes' transcription (Fischer and Hackl 2016; Koslová et al. 2024); meanwhile, sputnik uses viral RNA-processing proteins and amino-acyl tRNAs to drive its own protein translation. Hijacking of these viral components enables temporal activation of virophage gene expression specifically upon viral infection (La Scola et al. 2008; Fischer and Hackl 2016), which eventually results in dysregulated viral replication. It also explains why virophage production becomes spatially restricted within the viroplasm (La Scola et al. 2008; Fischer and Suttle 2011), promoting viral parasitism rather than the cellular host. Lastly, these virus-dependent mechanisms arguably allow the virophage to enter a quiescent, provirophage state in the absence (or upon clearance) of a viral infection, which disables

its parasitic properties that may affect cellular host functions (Fischer and Hackl 2016; Fischer and Suttle 2011).

Similarly, existing antiviral compounds like acyclovir are delivered as prodrugs, an inactive precursor of an antiviral compound that only activates in the presence of specific viral proteins. Viral thymidine kinase expressed by varicella-zoster virus or herpes simplex virus (Frobert et al. 2005) or phosphotransferase of cytomegalovirus (Talarico et al. 1999) converts acyclovir into its active state where the nucleoside analog binds to viral DNA polymerase and terminates DNA synthesis (Kausar et al. 2021), consequently inhibiting viral genome replication. This mechanism ensures specific activation of an antiviral system within infected cells, minimizing the negative effects of antiviral treatment on the uninfected cellular population.

Virophages can bind and take advantage of viral regulatory proteins due to their similar genetic makeup. However, it remains unknown how they can successfully hijack viral proteins or products, preferentially interacting with these molecules in a more kinetically favored manner. To investigate further, it is important to identify the viral and virophage molecules involved, determine active sites, and isolate relevant consensus sequences. Overall, this demonstrates a multi-faceted mechanism employed by virophages, particularly presenting an ideal antiviral strategy with spatiotemporal and viral protein-specific effects.

Abortive infection and inhibition of viral propagation

Viral infection may sporadically induce programmed cell death via abortive infection as part of virophages' antiviral system. Virophage-induced abortive infection among infected cells may seem counterintuitive as it triggers a molecular cascade that is both harmful to the virus and the cellular host. Nonetheless, mavirus demonstrates this tactic's effectiveness in clearing viral infection within a cellular population (Fischer and Hackl 2016; Koslová et al. 2024).

The utilization of antiviral drugs outside of medical therapy remains limited for several reasons. One major concern is their harmful effects on the host, necessitating controlled administration. However, antiviral systems that effectively induce abortive infection may also serve as a preventive measure in limiting viral infections transmitted by other biological vectors, such as insects. In the Philippines and other tropical countries, mosquito-related viral infections are a common seasonal concern (Victoriano-Belvis et al. 2021; Vista et al. 2020). The spread of *Filoviridae*, *Flaviviridae*, and arboviruses is commonly prevented using insecticides, which indiscriminately kills insects in a given area (Singh et al. 2008), consequently harming the natural ecosystem therein. Alternatively, the use of virophages or virophage-like strategies that selectively triggers an abortive antiviral system within infected cells would effectively target virus-containing insect vectors, which may even result in the development of insects' resistance against these viruses.

It has been hypothesized that mavirus triggers abortive infection as a strategy to not only inhibit viral replication, but also to release more virophage particles and limit subsequent viral propagation (Koslová et al. 2024). This displays an antiviral system that extends beyond the initial infection by providing an antiviral defense that significantly suppresses viral replication and propagation (Koslová et al. 2024). To our knowledge, there is no exogenous antiviral strategy that can inhibit viral replication and suppress propagation both at the initial and subsequent rounds of viral infection. Therefore, it would be beneficial to further investigate the specific mechanisms behind the parasitic and inhibitory properties of virophage against viruses. In this case, it may involve elucidation of the mechanisms behind abortive infection, specifically the molecular components that facilitate this process or its temporal activation that must occur post-assembly of virophage particles. Additionally, understanding mavirus' unique mechanism behind reducing subsequent infections may also reveal effective strategies that enable reduction of viral susceptibility and/or propagation.

It is worth noting that the intrinsic immune system can induce the release of signaling proteins, known as interferons. These biological molecules are released by infected immune cells after initial infection (Welsh et al. 2012). They signal neighboring cells to take on an antiviral state, which increases the expression of endogenous antiviral proteins, reducing cell susceptibility, and preventing subsequent infection by viral particles (Welsh et al. 2012). These immune response proteins, including antibodies, are used as passive and temporary immunization to neutralize and prevent viral propagation.

Other unique properties of virophages

Viruses are nature's most successful parasites. Besides their ability to hijack the cellular host and evade immune responses, they are also able to rapidly mutate and gain resistance to antiviral treatments. As viruses' natural parasites, virophages have the capacity to reliably persist and remain effective against their viral counterparts, unlike any existing antiviral therapy. Virophages can independently enter a cellular host, permanently reside within their host's DNA, replicate, propagate, and also mutate. Arguably, these properties align with the pseudo-living nature of viruses (Enespa et al. 2020), indicating a similar status for virophages. Fortunately, their genome is composed of a fundamental set of genes designed to specifically target and hijack a viral host. Therefore, fully elucidating the mechanisms behind virophages' parasitism against their viral hosts promise insights that can lead to the development of existing and novel antiviral therapeutics.

C. DISCUSSION

Currently, virophages are known to specifically parasitize giant viruses. These viruses independently encode for many enzymatic proteins and tRNAs that support cytoplasmic replication, an atypical characteristic for many viruses (Raoult et al. 2004; La Scola et al. 2008). Sputnik and mavirus have co-evolved with these viral hosts (Tokarz-Deptula et al. 2024) likely as an adaptive strategy that would facilitate faster virophage particle production - as parasite's parasite, rather than persisting as transposable elements within a cellular host. Although the existence of virophages that target disease-causing viruses is not unlikely, if one were to exist with similar characteristics as the ones described today, its direct utilization as treatment may become controversial due to various safety concerns (discussed further in section D: Scope and limitation). Regardless, virophages' characteristics as successful parasites against viruses demonstrate their potential to be a source or model for effective antiviral strategies.

In the Philippines, the search for novel antiviral compounds from natural resources is a significant field of study. Recently, many studies have tested various native medicinal plant extracts for antiviral properties against different viruses, *in vitro* (Victoriano-Belvis et al. 2021; Vista et al. 2020) and *in silico* (Cheng et al. 2022). In the same regard, the discovery of virophages provides another natural source of antiviral strategies and agents that may be useful in treating or preventing viral infections. The rich biodiversity and diverse ecosystems present

in our country make these types of research very promising. In fact, these have resulted in discoveries of novel viruses with unique characteristics (Kobayashi et al. 2017; Nabeshima et al. 2014); hence, it is also not unlikely to identify new virophages present in our ecosystems with distinct antiviral strategies. As such, this emerging field of antiviral research offers a promising path for expanding our virology research in the country.

The genetic material of virophages may also be used as blueprints for the emergence of novel antiviral gene therapy (Sankaranarayanan and Vishal 2017). Conceivably, genetically-modified virophages or virophage therapy may further open new avenues (Dutta et al. 2021) and strategies for eliminating viral infections that currently have no cure or treatment. Genetic modification of virophage regulatory elements may be used to redirect its parasitism against disease-causing viruses, selectively and dependently activating in the presence of virus-specific transcripts and/or proteins. For example, activation of HIV has been associated with TAT (trans-activator of transcription) viral protein binding onto TAR (trans-activation response), RNA elements found upstream of HIV transcripts (Jin et al. 2020; Laspia et al. 1989). If TAR elements were to be encoded in a virophage genome, it may be able to compete for TAT binding and induce virophage gene expression instead, resulting in viral inactivation and/or inducing abortive infection among infected cells. Alternatively, genetic modification may also be used to minimize or eliminate undesired virophage properties, such as genetic integration within the cellular host's genome or other currently unknown maladaptive effects. Although much research is still required, these types of modifications have the potential to generate effective and safe antiviral therapies.

Lastly, virophages' ability to co-evolve with their viral hosts presents a potential opportunity for antiviral therapeutics to keep up with the rapidly mutating viral genome. Selective pressure imposed by existing antiviral therapy onto viruses will inevitably result in antiviral resistance (Irwin et al. 2016; Frobert et al. 2005). Therefore, longitudinal research of virus-virophage interaction would have the potential to continuously elucidate new sources of antiviral strategies, as virophages can also mutate and adapt to antiviral resistance – a phenomenon known as evolution's 'red queen effect' (Serrano-Solis et al. 2018; Clarke et al. 1994). Overall, the discovery of virophages has arguably pushed the boundaries of what is possible when it comes to antiviral treatments and strategies. Characterization of their mechanisms opens a new avenue of research that holds great potential for elucidating effective antiviral strategies.

D. SCOPE AND LIMITATIONS

This narrative review highlights the antiviral properties of virophages, specifically focusing on described strategies employed by sputnik and mavirus virophages in parasitizing APMV/ACMV and CroV, respectively. While the precise mechanisms of these parasitic strategies are not yet fully elucidated, their existence hold tremendous value for antiviral therapeutics. As such, we also explore how specific virophage strategies compare to existing antiviral treatments and describe how further research may lead to new insights for the development of both existing and innovative therapies.

Precautiously, it is also important to note that virophages themselves are infectious. Although they require the presence of viral infection to activate, virophages utilize similar mechanisms as viruses for infection and replication within cellular hosts (Fischer and Hackl 2016). Additionally, some virophages, such as mavirus, integrate themselves into the cellular host's DNA, which may cause negative implications in the host cell's gene regulation and expression. Generally, the utilization of particles containing genetic material as a treatment is dangerous as they are susceptible to mutations that may potentially result in irreversible harm to the host (Bohne and Cathomen 2008). As such, this article does not recommend the direct usage of virophages as an antiviral treatment, at least without genetic modifications. It simply discusses their effective strategies to serve as potential models for the future developments of antiviral therapeutics. It is undeniable that these subviral particles have evolved a wide array of tactics in parasitizing viruses; as such, this paper aims to promote research into these mechanisms, which remain largely unexplored.

CONCLUSION

Virophages present a novel group of subviral particles with a unique set of parasitic strategies against viruses. Here, we outlined the strategic mechanisms employed by sputnik and mavirus in parasitizing APMV/ACMV and CroV, respectively (La Scola et al. 2008; Fischer and Hackl 2016; Koslova et al. 2024). Specifically, we explored the virus-dependent pathway of sputnik's entry into the cellular host, and its ability to hijack viral RNA-processing proteins and tRNAs to drive its own gene expression. Meanwhile, we also discussed mavirus' dependence on CroV's transcription factor/s in facilitating its transition from quiescent to active/enhanced state of transcription and its unique abortive infection strategy in propagating virophage particles, while limiting subsequent viral infections. Together, these strategies explain the parasitic nature of virophages against viruses, revealing some of their unique characteristics that includes spatial replication within the viroplasm and temporal activation specifically upon viral infection.

Virophages have emerged to be the parasite's parasite of nature. Their ability to hijack the viral machinery and limit viral replication demonstrate an array of effective and efficient antiviral systems against their host viruses. In fact, some of these strategies share similarities with existing pharmaceutical antiviral treatments, which we also highlighted in this review. We mentioned how selective cellular host-targeting, temporal- and spatial-specific activation, and abortive infection of virophages represent ideal properties for an effective antiviral treatment. Although the exact mechanism/s behind these strategies still lack, current literature has revealed important insights into existing and untapped antiviral mechanisms inherently present within the natural ecosystem. Therefore, understanding and fully elucidating virophage strategies against viruses offer significant promise for the future development of antiviral therapeutics.

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CONFLICT OF INTEREST

There is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

PVS was responsible for the conceptualization of the ideas, main manuscript writing, and editing. SGT was responsible for creating the figure and table, writing, editing, and referencing.

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