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Diversity of amoebal giant viruses isolated in China and intracellular interaction with host translation systems

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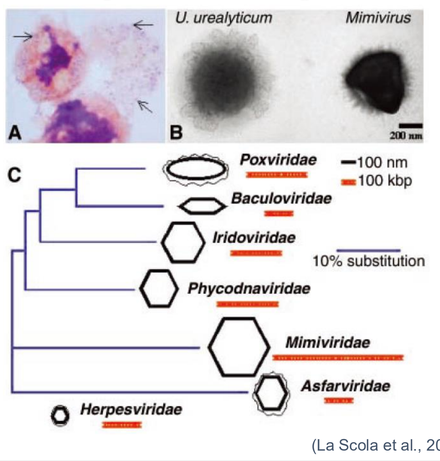
Abstract The discovery of amoebal giant viruses has blurred the boundary between virus and cellular life, since many virus genes never been described in other viruses. This study reports the isolation and characterization of novel amoebal giant viruses (Megavirus, Marseillevirus and Mollivirus) from various environmental samples in China. Key findings include the first identification of bacterial-like small multidrug resistance transporters in *Marseilleviridae*, suggesting potential roles in antibiotic neutralization. Furthermore, we uncover a divergence in translation termination mechanisms within *Marseilleviridae*, either by host factor mimicry or by autonomous viral complexes. These findings illuminate the remarkable evolutionary adaptability and unique host manipulation strategies of amoebal giant viruses, providing new insights into their environmental persistence and ecological significance.

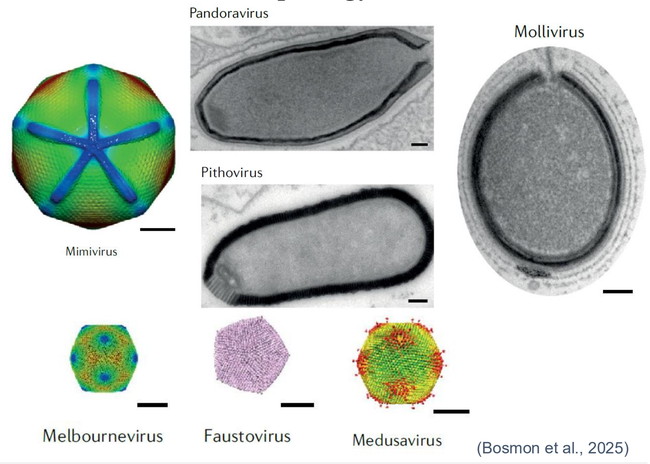
Introduction

First report of amoebal giant virus



(La Scola et al., 2003)

Diverse morphology of virions



(Bosmon et al., 2025)

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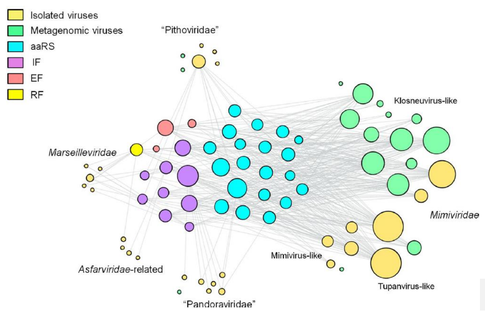

(Rodrigues et al., 2020)

Challenging the status quo: amoebal giant viruses and translation-related genes

Before the discovery of amoebal giant viruses, many scientists have considered viruses to the simplest biological entities, because of which components of the translational complex were not found in viruses.

Amoebal giant viruses and their complex genetic machinery is a milestone in modern virology, breaking a series of well established paradigms that have been in place since the 1950s, when André Lwoff postulated the principles for a biological entity to be considered a virus.

Figure 1. Diversity and connection of translation-related genes of amoebal giant viruses.

Methods

Amoebal giant virus isolation and purification

Water samples were collected from mangrove, river and pond, and then filtered through a membrane. The membrane was washed with Page's amoeba saline containing antibiotic cocktail. The elution of membrane was mixed with *Acanthamoeba castellanii*. Amoebal giant viruses are harvested after cell lysis and purified through a CsCl gradient from the cell debris.

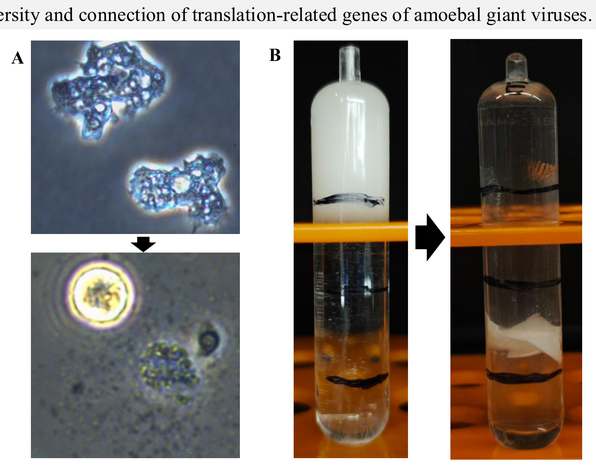

Figure 2. (A) the vegetative form of amoeba before and after infection of Marseillevirus. **(B)** Centrifuge tubes before and after separation of Marseillevirus particles on CsCl gradient.

Results

Overview of amoebal giant viruses

In this study, we isolated one new strain of Mollivirus, three new strains of Megavirus and eight new strains of Marseillevirus from diverse environmental samples in Guangdong and Zhejiang province, China.

Taken together with four earlier amoebal giant virus strains from Shanghai and Heilongjiang province, we established the first collection of isolated amoebal giant viruses in China.

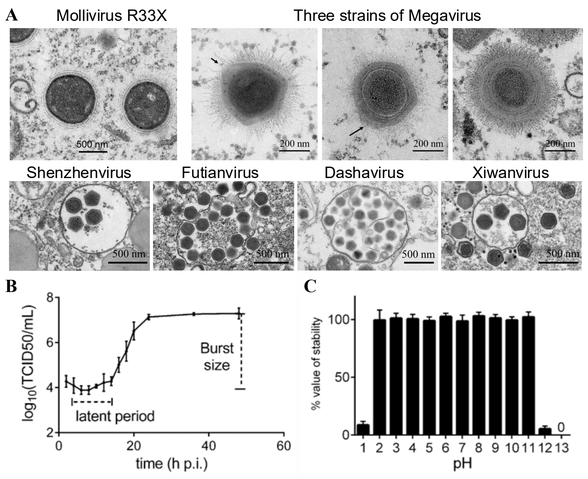

Figure 3. (A) Transmission electron microscopy image of Mollivirus, three Megaviruses and four Marseilleviruses in *A. castellanii* cytoplasmic. **(B)** The viral replication logarithmic curve of Mollivirus. **(C)** Histogram displaying the percentage of infectious Mollivirus viruses after treatments with PBS at varying pH levels.

Diverse transporter encoded by different amoebal giant virus

Among Marseillevirus genes without significantly similar sequences in databases and function unassigned, this is the first time that new diverse small multidrug resistance family of transporters (SMRs) are identified. Similarly, a major facilitator superfamily transporter (MFS) encoded by intron-containing gene is identified in Mollivirus.

The findings of viral SMRs and MFS indicate the potential interaction between amoebal giant virus and chemical components and shed insight into viruses' fitness of intracellular environment.

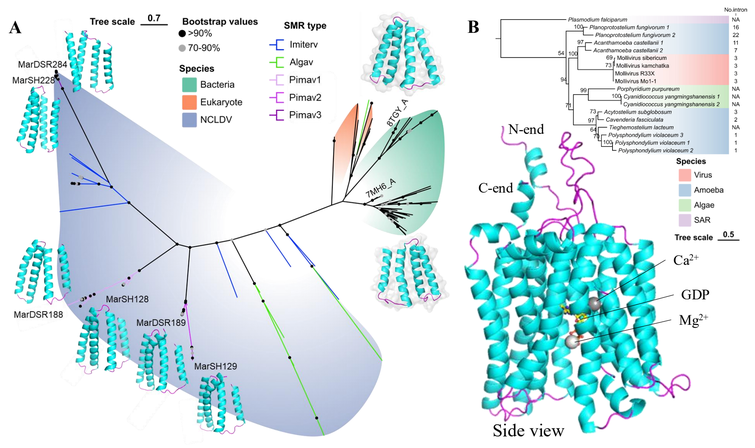

Figure 4. (A) Molecular phylogenetic analysis of Marseillevirus SMR family of transporter. The tertiary structures of bacterial and viral SMR are attached to the leaf names and the crystal structures are highlighted with grey surface covered. **(B)** Molecular phylogenetic analysis of eukaryotic and viral MFS followed by *in-silico* tertiary structures of the complex of Mollivirus MFS with ions and ligands.

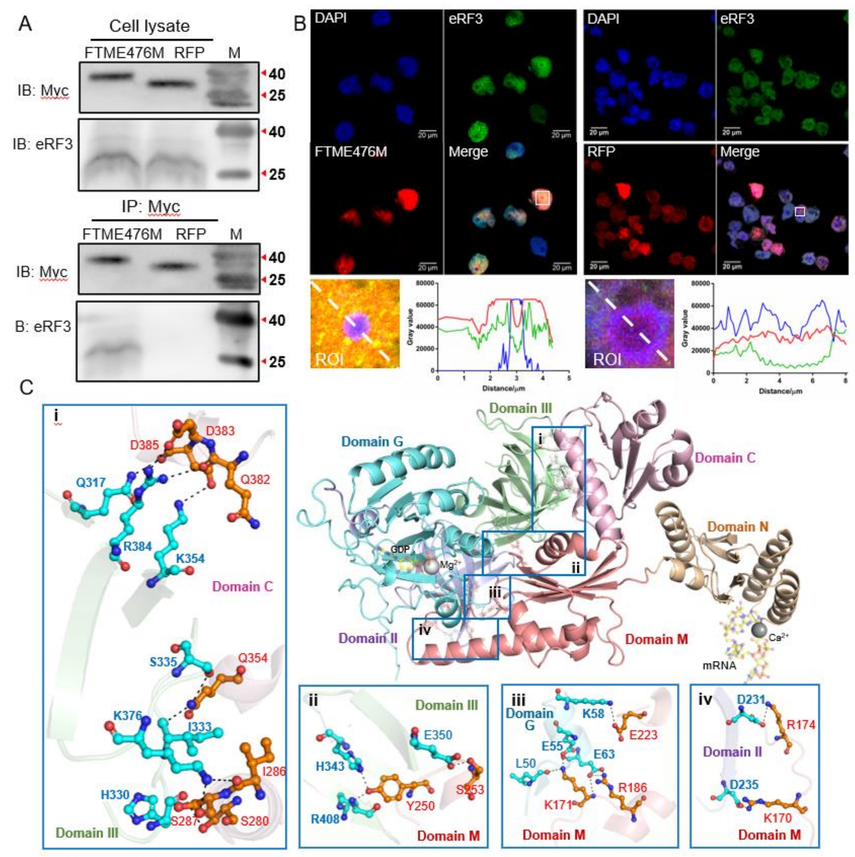

Figure 5. Interaction between Shenzhenvirus vRF1 (FTME476M) and host eRF3. **(A)** Western blotting analysis. Top: cell lysates from *A. castellanii* expressing FTME476 or RFP control, Bottom: co-immunoprecipitation assays in *Ac*-FTME476M with anti-Myc antibody and anti-eRF3 antibody. **(B)** Immunofluorescence analysis. *Ac*-FTME476M cells stained with anti-Myc antibody and anti-eRF3 antibody. **(C)** Predicted structural model of the FTME476·eRF3 termination complex.

Shenzhenvirus vRF1 recruits host eRF3 to form the viral–host termination complex

Given the absence of vRF3 in Shenzhenvirus, we hypothesize that its vRF1 (FTME476) recruits host eRF3 to form a hybrid termination complex. To test the hypothesis, we employe a comprehensive multi-step approach including co-IP coupled with LC-MS/MS, Western blotting and immunofluorescence analysis. We observe co-localization of FTME476 and eRF3, as evidenced by the merged co-localization signal within the cytoplasm. Such a co-localization signal is not present in cells expressing RFP, confirming the specificity of the FTME476·eRF3 interaction.. Additionally, *in-silico* structural modeling of the FTME476·eRF3 complex predicts four interfacial contact regions, mirroring eukaryotic eRF3·eRF1 complexes.

Host-dependent mimicry and autonomous strategies

- In vRF3-deficient viruses, vRF1 recruits host eRF3 for peptidyl-tRNA hydrolysis.
- Similarly, in vRF1-deficient viruses, vRF3 recruits host eRF1.
- In vRF-sufficient viruses, autonomous viral complex terminates translation directly

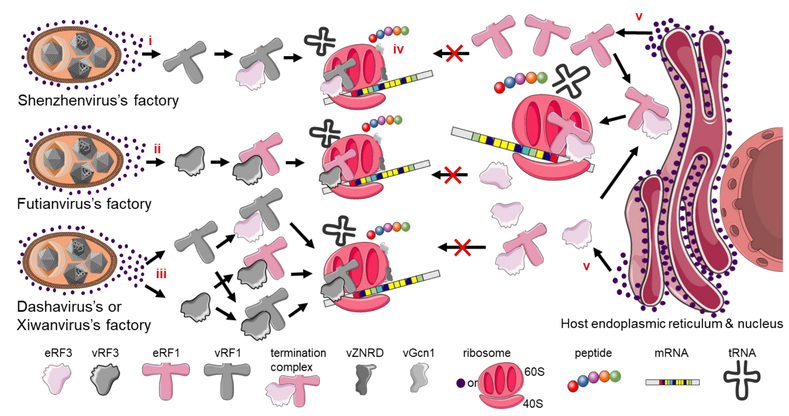

Figure 6. Schematic models depict diverse termination strategies by different marseillevirus.

Conclusions This work significantly expands our understanding of amoebal giant virus biology. The isolates demonstrated robust and various environmental adaptability, while the discovery of homologous transporters highlights convergent evolution in manipulating host interactions. The diverse translation termination strategies in *Marseilleviridae* underscore an unexpected level of evolutionary plasticity in viral translational control. Collectively, these findings highlight amoebal giant virus unique evolutionary trajectory and adaptive strategies for survival. Future research would focus on experimentally validating the functions of these identified viral genes to further elucidate their roles in infection and ecology.

Acknowledgements

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