Structural insights into viral RNA capping and plasma membrane targeting by Chikungunya virus nonstructural protein

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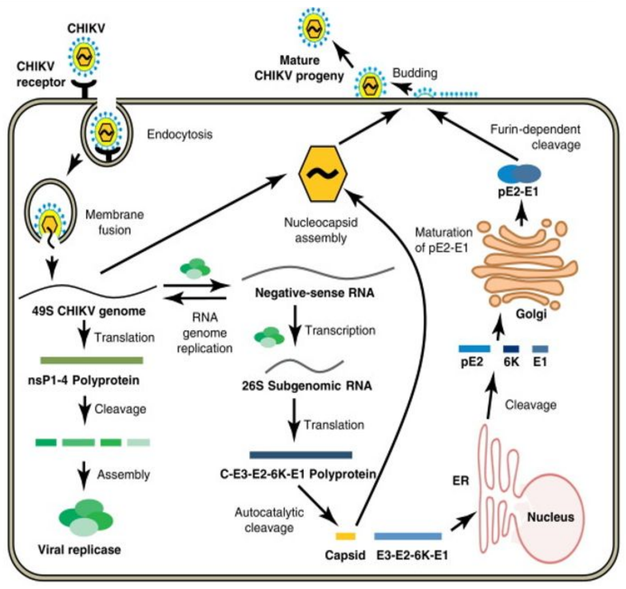
Introduction

- With no vaccine or antiviral available, Chikungunya virus (CHIKV) is an emerging global healthcare crisis.
- The protein of interest encoded by the CHIKV genome is nsP1.
- This paper aims to determine the structural basis of nsP1's role in viral replication (right).



Methods

Step 1

Recombinant nsP1 proteins were purified from *E. coli* and Expi293F human cells.

Step 2

nsP1 proteins were examined using analytical size-exclusion chromatography (SEC) and negative-staining EM.

Step 3

A gel-based MTase/GTase assay was performed to determine which nsP1 proteins had mRNA capping activity.

Step 4

nsP1 structure was experimentally determined using single-particle cryogenic electron microscopy (cryo-EM) at 2.38Å.

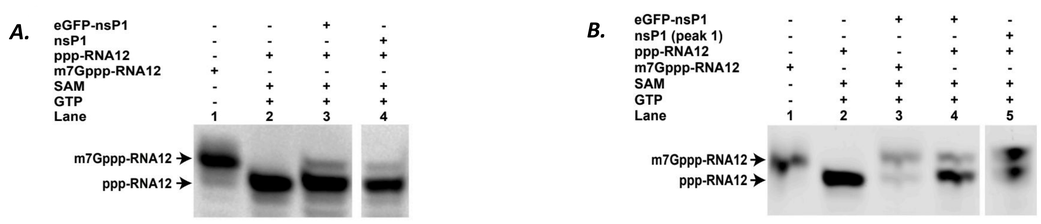
Results & Discussion

Analytical size-exclusion chromatography and SDS-PAGE analysis

Recombinant nsP1 proteins were purified from *E. coli* and Expi293F human cells. Recombinant proteins from Expi293F were eluted as a single high molecular weight ring-shaped complex while proteins from *E. coli* were eluted over a wide range of molecular weight with 3 distinct peaks using size exclusion chromatography (SEC). This was supported by SDS-PAGE analysis of the proteins as well.

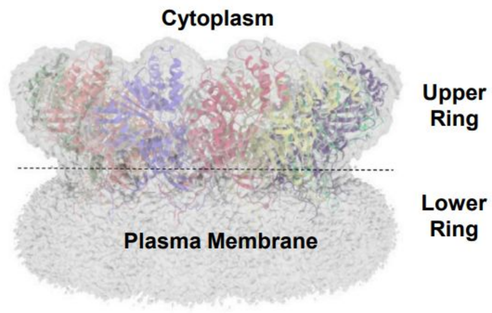
MTase/GTase assays

From *Figure A,* both nsP1 proteins from the Expi293F and the bacterial nsP1 from the high MW peak were active, evidenced by presence of guanylyltransfer onto substrate RNA in lane 3 and 4 respectively. Further exploration of the various nsP1 proteins is shown in *Figure B.* nsP1 purified from Expi293F in lane 3 was found to behave more similarly *in vivo* and is therefore preferred in this study.



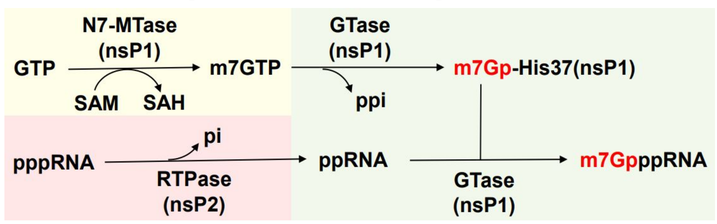
General structure of nsP1

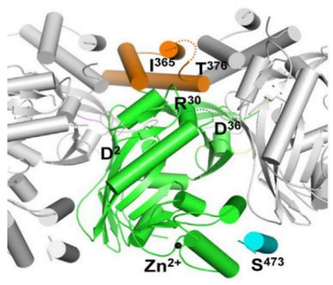
The CHIKV nsP1 structure comprises 12 copies of nsP1 arranged in a dodecameric ring of perfect C12 symmetry. The ring structure can be divided into upper and lower portions *(right).*



Role of nsP1 in Viral RNA Capping

The upper portion of the nsP1 ring is made of the dual-functional MTase/GTase catalytic domain that adds cap-0 structure to newly formed viral genomic and subgenomic RNA transcripts during replication. The capping process is shown in the equation below:

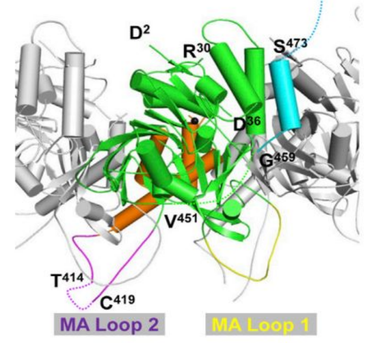




The nsP1 MTase/GTase core domain shares structural homology with the mRNA N7-MTases of eukaryotic and dsDNA viruses, as well as to a lesser extent in other (+) RNA viruses such as coronavirus and flavivirus, indicating the evolutionary conservation of SAM-MTases. However, despite the common homology, the structural basis and the catalytic mechanism of CHIKV nsP1 GTase are different from the functionally equivalent non-alpha viral GTases.

Role of nsP1 in Plasma Membrane Targeting:

The lower portion of the nsP1 ring is the membrane-association (MA) domain, which is essential for virus replication. This process is mediated mainly by two intertwining MA Loops 1 and 2. Membrane association is achieved via the direct insertion of the palmitoylated triple cysteine motif and the hydrophobic residues. Membrane association is not only key in virus replication, but also promotes the catalytic activities of nsP1. Therefore, nsP1 is likely to serve as the scaffold for the viral replication complex assembly, which includes the viral RNA template, the other nsPs, and accessory host factors.



Conclusion

- In summary, high-resolution cryo-EM visualisation of the CHIKV nsP1 dodecamer ring structure has improved understanding of the viral RNA capping process & formation of the membrane-associated replication complex.
- However, more research is required in determining the architecture of the replication complex and understanding the spatiotemporal regulation at different steps of viral RNA replication.

References

Zhang, K., Law, Y.-S., Law, M. C., Tan, Y. B., Wirawan, M., and Luo, D. (2021). Structural insights into viral RNA capping and plasma membrane targeting by Chikungunya virus nonstructural protein 1. Cell Host & Microbe *29*, 2061–2063.

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