Probing the origin of RNase resistance in viral xrRNAs

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Abstract

DNA and RNA are very important building blocks to all life at the molecular level. All known organisms' genetic instructions for development, function, and reproduction are stored in DNA. RNA is also a nucleic acid and has similar structures to DNA, and is essential for protein synthesis. It acts as a messenger and copies genetic instructions from DNA to translate into proteins. RNA is especially important to most viruses because it carries the genetic information. In the current project we are working on West Nile Virus(WNV), a member of the Flavivirus family. Flaviviruses are a group of viruses that have single stranded RNA genomes that directly translate to viral proteins. These viruses are found to have uniquely folded RNA that prevents breakdown by exoribonucleases: enzymes that digest viral RNA in the body. A previous study done on Zika virus has shown exoribonuclease mediated digestion which leads to accumulation of smaller fragments in the host. The RNase enzymes that help defend the cell against invading viral RNA are used to generate fragments that help the virus infect the cell.These smaller fragments lead to apoptosis and cell death. My research involves studying West Nile Virus and it mutants to research the regions involved in exoribonuclease digestion. I did molecular biology work that involved introducing mutations in DNA, modifying DNA by putting promoter sites to make RNA, and finally RNA synthesis.

Background and Motivation

- The unique structure in flaviviruses prevent complete degradation of viral genomic RNA
- This leaves small RNA fragments that attach anywhere in the body, which enhances viral replication and infection

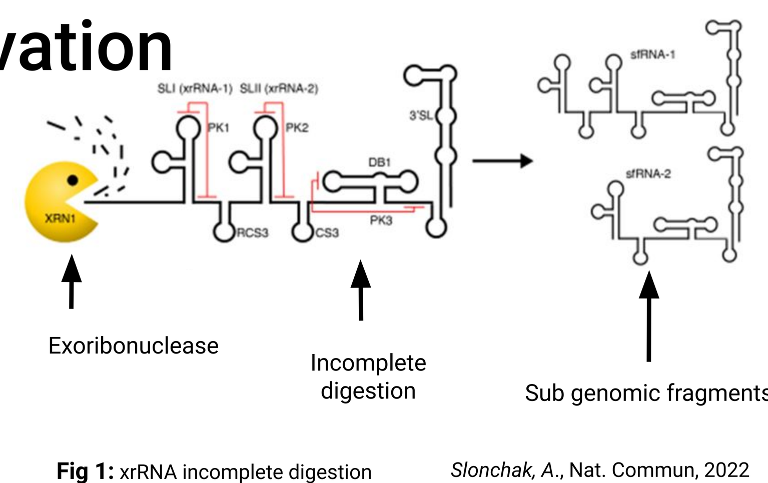


Fig 1: xrRNA incomplete digestion *Slonchak, A., Nat. Commun, 2022*

- **Goal is to figure out which interactions in the xrRNA structure are essential for RNase resistance and be able to disrupt those interactions when developing potential drugs**

Experimental Methods

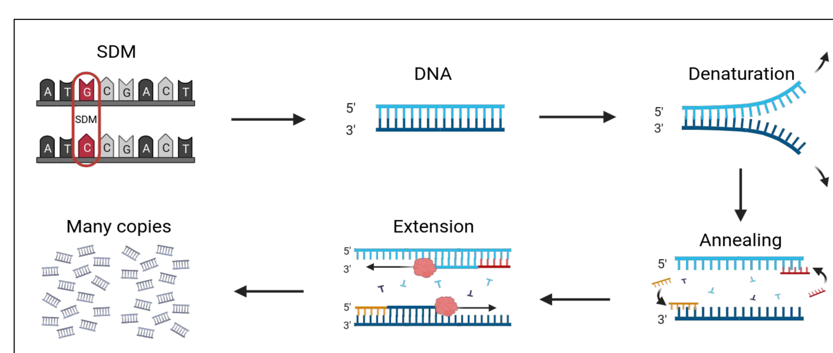


Fig 2: SDM and PCR process, [BioRender object]

- **Site Directed Mutagenesis(SDM) and Polymerase Chain Reaction (PCR)** - To study the regions affected by the exoribonuclease digestion, a specific base in the DNA is changed (SDM) and many copies of the DNA are made through PCR
- **Quantifying DNA with agarose gel electrophoresis** - Running an electric current through the gel to separate DNA fragments by length
- **Bacterial transformation** - Introduce our DNA into bacteria and grow bacterial colonies to clone the DNA
- **Purification of DNA and In-vitro RNA synthesis** - From the cell, isolate our plasmid and use to set up transcription templates to synthesis RNA for further study

Results

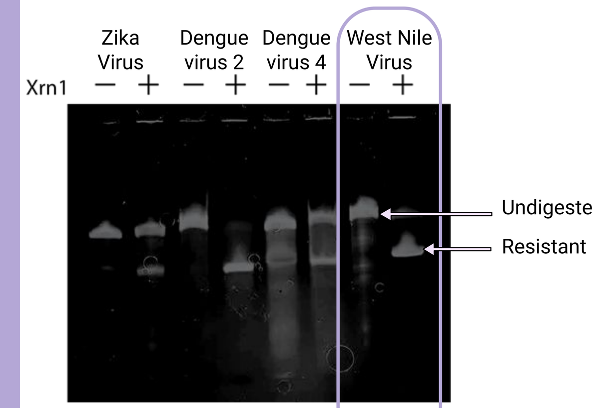


Fig 3: Digestion of viral RNA Gel based assay

- This gel shows the result of exoribonuclease mediated digestion in these flaviviruses
- Two samples of each virus was run, - is without enzyme digestion, + is with enzyme digestion
- The lower band shows positive results because the enzyme digested the virus into smaller fragments that migrate farther down the gel.
- **My research is focused on WNV and its mutants**

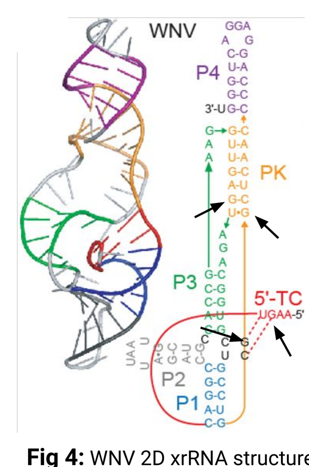


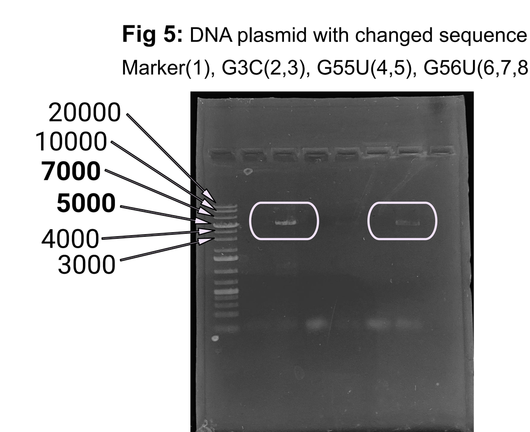
Fig 4: WNV 2D xrRNA structure

- Three single mutations introduced: G3C, G55U, G56U
- One double mutation introduced G56U | G37C

SDM and PCR

- The bands show the molecular weight of the sample and that the DNA has properly multiplied
- The two bands shown are between 7000 and 5000 base pair length (bp length) according to the marker

Fig 5: DNA plasmid with changed sequence Marker(1), G3C(2,3), G55U(4,5), G56U(6,7,8)



Bacterial Transformation

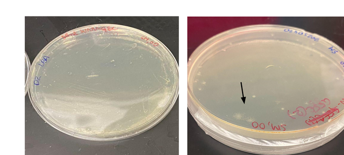


Fig 6: Bacteria colonies of WNV G3C mutant

- After DNA is amplified, it is introduced into bacteria to grow colonies and clone itself
- Select colonies are picked up to purify to test the DNA sequence and for RNA synthesis

DNA Sequencing

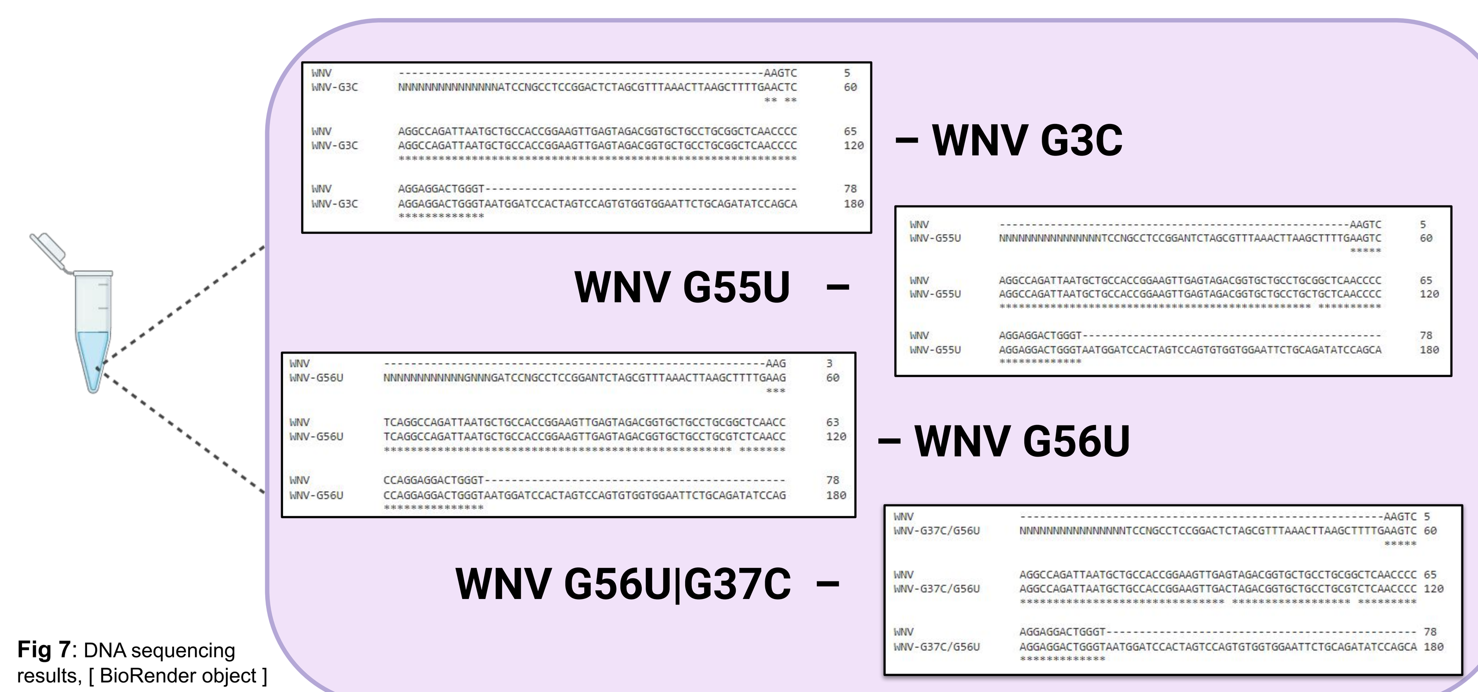


Fig 7: DNA sequencing results, [BioRender object]

- All the stars show that our DNA sequence exactly matches the parental strand except for the one spot with the mutation
- Once sequencing was correct, RNA was synthesized and purified

Summary

- The resistance of exoribonuclease in flaviviruses prompted the research on the regions involved in the digestion
- All four mutants, G3C, G55U, G56U, and G56U | G37C were successfully done and amplified
- The DNA of the bacterial colonies was purified and from that, RNA was synthesized and purified

Discussion - Further Work

- Zika, Dengue isoforms 2 and 4, and West Nile all undergo exoribonuclease mediated digestion
- Due to the constriction of time, I did molecular biology work and generated WNV mutants and synthesised RNA which will be used to do gel based assay
- The gel based assay will show if the exoribonuclease will fully digest the virus or if it will still digest into smaller fragments
- This will provide more information on the structural regions responsible for the resistance

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