Mapping of a Classical Temperature-Sensitive Replicase Mutant of Mouse Hepatitis Virus

Terry Hafer 1,2  And Dr. Paul Masters, PhD 1

1Wadsworth Center, New York State Department of Health
2Pennsylvania State University

Abstract

Coronaviruses are positive-sense RNA viruses that can cause human Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS). Mouse Hepatitis Virus (MHV) is the prototype used here to analyze temperature-sensitive (ts) mutants of Alb2 that can grow at 33°C, but not 39°C. Previous results suggested that the mutation in Alb2 may occur within nonstructural proteins nsp1-11 of the 16-subunit replicase gene [1]. Analysis of the ts mutant Alb2 using RT-PCR and sequencing showed that Alb2 has a point mutation in the Y domain of nsp3 resulting in an amino acid change from Alanine to Valine. In addition, Alb2 was found to have a 47-nt deletion and a point mutation in the 3’UTR. Six revertants were selected for further analysis. One revertant analyzed from nsp1-11 was found to have a single primary site mutation in the Y domain of nsp3 causing it to revert to wild type MHV. Those remaining were sequenced only in the nsp3 region and all reverted back to WT. No second-site mutations were found in any of these six, and the 47-nt change and the point mutation in the 3’ UTR were all maintained in them. The results of revertant analysis strongly suggest that the mutation responsible for temperature-sensitivity in Alb2 is the point mutation in the Y domain of nsp3. To complete the analysis of ts mutant Alb2, the remaining genome will be sequenced to determine if other mutations downstream of nsp11 might impact the Y domain of nsp3.

Materials and Methods

- The ts Alb2 mutant was sequenced from nsp1-11 (12,000 bp) to find mutations.
- Infect mouse cells with Alb2 mutant
- Isolate RNA
- Generate cDNA
- Amplify through PCR
- Send for sequencing; Compare sequences of Alb2 mutant with wt.
- Generate revertants for revertant analysis
- Sequence viral RNA of each revertant using the same process as in the ts Alb2 mutant, and search for primary or secondary site mutations

Results

- Infected flask was initially at the permissive temperature (33°C), but then shifted to the nonpermissive temperature (39°C) in order to select for revertants. Plaque purification was done by infecting cells with the virus on a cell/culture dish. The infection was overlaid with agarose and growth media.

Conclusions

- Alb2 has the following mutations:
  - Point mutation in the Y domain of nsp3
  - Point mutation in the 3’UTR
  - 47 nucleotide deletion
- The results of the revertant analysis strongly suggest that the mutation responsible for temperature sensitivity lies in the Y domain of nsp3.
- The deletion and point mutation in the 3’UTR are not responsible for temperature sensitivity in Alb2.
- The N-terminus of nsp3 interacts with the N protein, and has been shown to be indispensable to the virus, but little is known about the Y-domain and its function [2].
- Further work must be done to understand the role of the Y-domain of nsp3.

Future Work

- Sequence the rest of the genome – are there any more mutations?
- By reverse genetics, change amino acid 1946 of nsp3 to a different amino acid that is similar to Valine.
- Isolate revertants at 37 degrees rather than 39 degrees
- This may show how different proteins or different parts of nsp3 are interacting with the Y-domain.

Acknowledgements

I would like to acknowledge Dr. Jan Keithly and Dr. Matt Kohn for organizing this REU program. I would like to thank all of the members of the Masters lab, Dr. Paul Masters, Dr. Lili Kuo, Cheri Koetzner, and Kelley Hurst. Furthermore, I would like to especially recognize Dr. Lili Kuo and Cheri Koetzner for working closely alongside me throughout this process. In addition, I would like to thank Dr. Lawrence Sturman for isolating tsAlb2, and the Wadsworth sequencing facilities for returning sequences in a timely manner. Finally, I would like to thank the National Science Foundation (NSF) for funding in this summer REU.

References


Objectives

- Find the mutation responsible for the temperature-sensitivity phenotype in Alb2.
- Understand how revertant viruses can mutate to compensate for the temperature-sensitivity.