

The Role of the RCK/p54 Nuclear Localization Signals on Hepatitis C Virus Infection

Hepatitis C virus (HCV) is a predominantly blood-borne virus that affects approximately 3.2 million persons in the United States. Those infected are at a heightened risk for cirrhosis of the liver and hepatocellular carcinoma. Although the new antiviral drug treatments targeting the viral protease and polymerase hold much promise for a cure, our understanding on the virus and the interactions with the host cell is incomplete.

HCV gene expression and virus assembly, as discovered by the Payer lab, requires RCK/p54 during infection. RCK/p54 is a DEAD-box helicase involved in microRNA gene regulation, translational repression, and mRNA storage and decay. RCK/p54 contains a lysine-rich nuclear localization signal (NLS) and a leucine-rich nuclear export signal (NES) localized at the N-terminal region. Both the NLS and NES allow RCK/p54 to be shuttled between the nucleus and cytoplasm. I hypothesized that the shuttling of RCK/p54 plays a role in HCV infections. To test this hypothesis I created mutations at these sites to disrupt the ability of RCK to shuttle across the nuclear membrane. My data demonstrated that mutating the NLS did not affect RCK/p54 expression. Interestingly, mutant NLS RCK/p54 migrated in a denaturing polyacrylamide gel higher than the wildtype protein, raising the possibility that the mutant protein might be post-translationally modified. Additionally subcellular fractionation showed that the mutant NLS RCK/p54 protein, as expected, is localized in the cytoplasm. These studies have established the ground work to examine the role of the RCK/p54 nuclear localization signals during HCV infection.

Keywords: Hepatitis C Virus, RCK/p54, DEAD-box helicase, Nuclear Localization Signal, Huh7.5 Cells