Single Molecule Mechanistic Studies on Polymerase Activity

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The hepatitis C virus (HCV) is recognized as major human pathogen. Approximately 250000 infected individuals live in Canada. HCV infection is associated with severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Unfortunately, the availability of potent inhibitors that block replication of HCV is limited, and by far not everyone benefits from therapy with pegylated interferon-alpha and ribavirin. These drugs are not HCV-specific. Severe toxic side effects, the ability of the virus to evade the host interferon system, and the enormous genetic variability of the virus are recognized as factors associated with treatment failure. A better understanding of specific steps involved in HCV RNA genome replication will likely improve the basis for the development of novel antiviral drugs with improved potency and specificity.

Here we describe experiments that are designed to: 1) gain a molecular level understanding of de novo initiation of RNA synthesis catalyzed by HCV and 2) elucidate the functional roles of the GTP binding sites in HCV. We have used Single Molecule and ensemble fluorescence resonance energy transfer, as well as fluorescence polarization studies to address the interactions between HCV and substrate oligonucleotides. We will describe these preliminary results in the presentation.