#61: Molecular Mechanisms of Hepatitis B Virus Covalently Closed Circular DNA Formation
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Hepatitis B virus (HBV) remains a major human pathogen causing acute and chronic viral hepatitis, liver cirrhosis, and cancer. As a prototypic hepadnavirus, HBV contains a small, partially double-stranded, relaxed circular (RC) DNA genome. Early during viral infection, RC DNA from the incoming virion is converted to the covalently closed circular (CCC) DNA, which can also be formed from progeny RC DNA synthesized de novo in cytoplasmic viral nucleocapsids (NCs). CCC DNA functions as the only viral template capable of coding for all the viral RNA species and is thus essential to initiate and sustain viral replication. Current antiviral therapies can suppress viral replication but cannot eliminate CCC DNA, the persistence of which remains a major obstacle toward curing chronic HBV infection. Currently, little is known about how CCC DNA is formed due to the lack of convenient experimental systems that can support efficient CCC DNA formation. For CCC DNA formation, RC DNA must be released from viral nucleocapsids into the host cell nucleus. The disassembly process of nucleocapsids (i.e., uncoating) required for RC DNA release is not understood.

We have recently shown that mutations of the viral capsid protein can lead to enhanced CCC DNA formation by stimulating NC uncoating. Furthermore, we have identified host cells with enhanced CCC DNA formation also via the stimulation of NC uncoating. These capsid mutants and host cells now provide native RC DNA substrates readily accessible for CCC DNA formation, which is being attempted under cell-free conditions with the help of cell extracts. As the viral polymerase protein remains covalently attached to RC DNA as a result of protein-primed initiation of viral DNA synthesis, its removal constitutes an essential step in the conversion of RC to CCC DNA. We and others have recently shown that a host cell DNA repair enzyme, the tyrosyl DNA phosphodiesterase 2 (Tdp2), is able to specifically cleave the polymerase-RC DNA linkage in vitro. However, the role of Tdp2 in CCC DNA formation in vivo remains controversial and seems to be opposite for HBV vs. its avian counterpart, the duck HBV (DHBV).

We are examining further the role of Tdp2 in RC DNA deproteination and CCC DNA formation in vivo, as well as the role of other host factors in this and other steps of CCC DNA formation. In order to elucidate the pathway(s) of CCC DNA formation from RC DNA, we are also attempting to isolate intermediate DNA species during the conversion process.