

HEPATITIS B VIRUSES: THE GYMNASTICS OF GENOME REPLICATION

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Hepadnaviruses are a family of hepatotropic DNA viruses that replicate their genomes through reverse transcription of an RNA intermediate called pregenomic RNA (pgRNA). Hepatitis B virus (HBV), the prototype member of this family, chronically infects ~350 million worldwide and leads to a variety of liver diseases including hepatocellular carcinoma. The process of reverse transcription involves the stepwise conversion of pgRNA into a single-stranded minus-strand DNA that is then converted into a double-stranded relaxed-circular (RC) DNA, the form of the genome found in infectious virions. Like other reverse-transcribing entities, hepadnaviruses use a series of complex and dynamic template switches during genome replication to ensure synthesis of the correct end-product. Genome replication/reverse transcription occurs within the viral nucleocapsid in the cytoplasm of the initially infected cell. We use HBV and its related cousin, duck hepatitis B virus (DHBV), to study genome replication/reverse transcription.

A major theme emerging from our work is that hepadnavirus genome replication requires complex contributions from the replication template. The genomic RNA and DNA replication intermediates are not passive templates, but proceed through dynamic topologies that guide their own replication. We have three types of results that illustrate this theme. First, we found that long-range base pairing in pgRNA makes multiple contributions to replication. DHBV pgRNA adopts a conformation to prevent it from being spliced and promotes its accumulation for genome replication. Also, we found that long-range base pairing of HBV pgRNA promotes synthesis of minus-strand DNA, presumably during template switching. Second, we found that long-range base pairing in the minus-strand DNA makes contributions to both template switches (primer translocation and circularization) during RC DNA synthesis for both DHBV and HBV. Third, for DHBV we found that a small DNA hairpin in the minus-strand DNA template regulates the selection of the proper site of initiation of synthesis of plus-strand DNA.