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Hepatitis B Virus Replication by Core and Polymerase Proteins and its Modulation by Splicing

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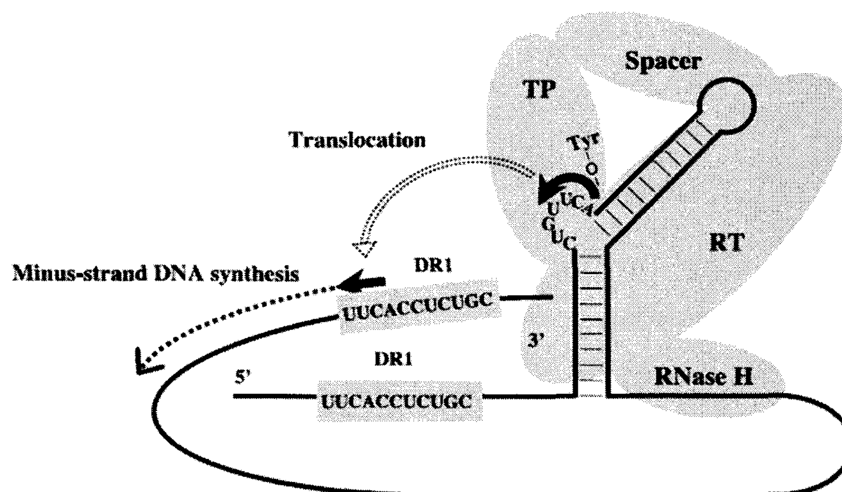
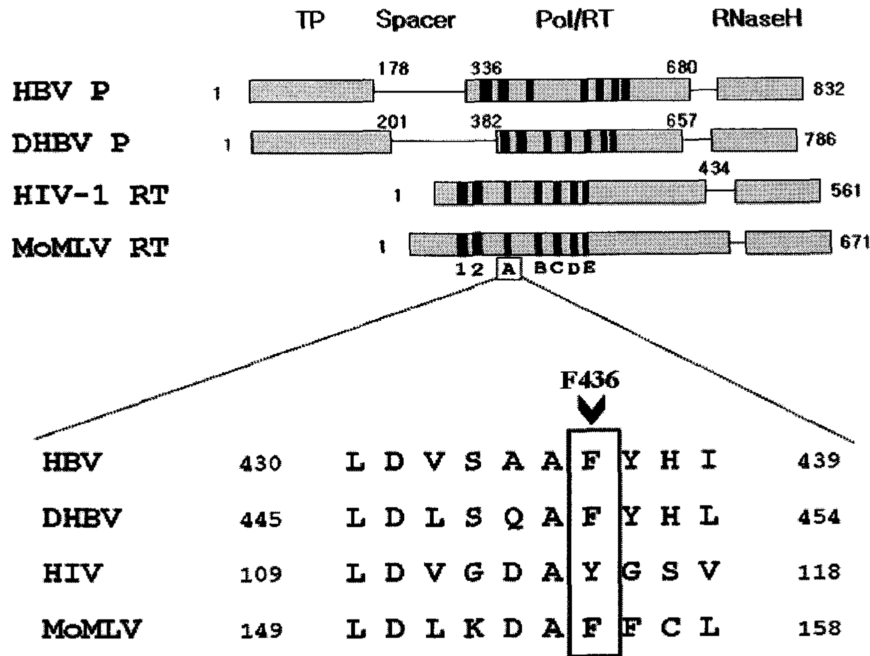
Assembly of the replication competent HBV core particles requires interaction of core (C) and polymerase (P) proteins and pregenomic RNA (pgRNA). Hepadnavirus DNA P protein functions in DNA synthesis and encapsidation, and acts as a primer for minus-strand DNA synthesis.

Through protein priming reaction, a short DNA oligomer synthesized from the bulge of epsilon (ε) as a template, is covalently attached to the Tyr residue in the terminal protein (TP) domain of DNA P protein. Using endogenous polymerase assays and native agarose gel analysis, we detected endogenous polymerase activity in priming-deficient mutant core particles, but not in RT reaction- or P protein-deficient mutant core particles. We found that the priming-deficient mutant P protein has the ability to synthesize oligomers (presumably nascent minus-strand DNA) in the absence of the primer. We propose that the priming-deficient mutant may be defective in minus-strand DNA translocation to direct repeat (DR) 1 at the 3'-end of pgRNA that leads to the elongation of minus-strand DNA.



This primer independent initiation by priming-deficient mutant P protein raises a very important question that HBV DNA polymerase may have RNA polymerase feature. So, the RNA polymerase

activity of HBV P protein was explored by testing the NTP incorporation capacities with F436G and F436V mutant P proteins. These mutant P proteins incorporated NTPs by endogenous polymerase activity, indicating that properties of DNA and RNA polymerases are blurred bysingle amino acid substitution.



To identify the motifs of HBV C protein that are necessary for HBV replication and the nucleic acid binding domain, chimeric C proteins of HBV and duck HBV (DHBV) were analyzed. We found that 40% of amino acid sequence identity of C-terminus of chimeric C proteins at nucleic acid binding domain is sufficient to complement HBV C protein for HBV replication, such as core particle assembly, pgRNA encapsidation, and/or HBV DNA synthesis.

Spliced transcripts in HBV infected liver and HBV-transfected hepatoma cells are not essential for HBV replication, but the functions and associations with chronic hepatitis and hepatocellular carcinoma are not clearly elucidated yet. The polymerase-surface (PS) fusion proteins from spliced transcripts were co-localized with nuclear pore complex, vimentin, and endoplasmic reticulum in perinuclear region. Hepatitis B surface antigen secretion, core particle formation, and HBV DNA synthesis were drastically inhibited by expressions of PS RNA and PS protein or, to a lesser extent, PS RNA only. Taken together, we suggest that splicing might be one of the consequences of host-virus interactions to modulate virus replication or to survive through virus infections, as possible implications to persistent HBV infections and carcinogenesis.