E313 Structure-Function Analysis of HBV polymerase

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Hepatitis B Virus (HBV) polymerase replicate(s) its genome through the self-priming using terminal protein, strand switching, elongation (and so on). To investigate the complex replication mechanism of HBV polymerase *in vivo*, the trans-complementation of the mutated HBV genome containing the frame-shift mutation in polymerase gene with the expression constructs of serially deleted mutant polymerases were performed. None of the deleted polymerase could trans-complement the mutated HBV genome. This indicates that even the short fragments of the N-terminal(1-82) and C-terminal(778-842) are required for the HBV genome replication. To investigate the interaction site of POL protein with HSP90, co-immunoprecipitation experiment was performed. The result showed that 97-212 and 538-680 of pol protein interact with HSP90 protein. Further trans-complementation experiments showed that 200-262 amino acid region of HBV polymerase is not essential for replication and 262-336 amino acid region of polymerase interacting with terminal protein directly play a role in replication.

E314 Fidelity of DNA Synthesis by Human Hepatitis BViral Polymerase

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Hepadnaviruses replicate their nucleic acid through a reverse transcription step. Recenty, we expressed MBP-fused HBV polymerase in *E. coli* and purified by amylose affinity column chromatography. The purified protein shows DNA-dependent DNA polymerase activity. In this report, we analyzed the fidelity of the DNA-dependent DNA polymerase activity of the HBV polymerase and compared it with that of HIV and MMLV RT. Like other retroviral RTs, the HBV polymerase was shown to lack 3->5 exonuclease activity. The ability of HBV polymerase to incorporate wrong nucleotide was examined by quantitating the amount of incorporated band by phosphoimager system. The results demonstrate a relatively efficient nucleotide incorporation by the HBV polymerase with a specificity of three groups: (1) A:T, T:A> C:G, and G:C (matched pairs), (2) A:C, C:A> G:T, T:G (purine-pyrimidine mispairs), and (3) T:T, A:A, G:G> T:C, C:T> A:G, G:A (purine-purine and pyrimidine-pyrimidine mispairs), and the order of specificity is (1)>(2)>(3). Fidelity of nucleotide insertion by the HBV polymerase was examined and the results suggest that the HBV polymerase is as error-prone as HIV-1 RTs.