SL306

FUNCTIONAL MAPPING OF THE RT DOMAIN OF THE HBV REVERSE TRANSCRIPTASE : TRANSCOMPLEMENTATION OF NUCLEOTIDE PRIMING AND REVERSE TRANSCRIPTION

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We have previously described the purification of a functional human HBV Pol following expression in insect cells from recombinant baculoviruses. Both the TP and RT domains were required for in vitro nucleotode priming. In an attempt to establish a complementation system for analysis of the roles of TP and RT in the primig reaction, we evaluated transcomplementation between TP and RT mutants, Although full length Pol proteins with mutations in TP and RT were incapable of complementing each other in the priming reaction, TP and RT could be expressed independently and still form a RNP complex with epsilon that was capable of in vitro priming reactions. We have prepared baculoviruses expressing amino and carboxyl terminal deletions of both the TP and RT domains and have analyzed the proteins for the ability to form complexes functional in the priming reaction. The minimal TP domain spanned amino acids 20-175; however, very little activity was observed without a TP domain spanning amino acids 1-199. Thus the TP expends into the spacer domain. The minimal RT domain spanned amino acids 300-775; however little activity was observed unless the carboxyl end of the RT domain extended to amino acid 800. Thus, the RT domain required for transcomplementation begins within the spacer domain and extends through most of the RNaseH domain. Previously, we observed that a TP protein with a carboxyl terminus at amine acid 334 was not functional. We hypothesized that an inhibitory domain was present between amino acids 199-344. The deletion analysis narrowed this domain to residues 300-334 which is a portion of the minimal RT domain. It is possible that the presence of a portion of the RT domain within the TP protein permits intraprotein interactions that inhibit appropriate interprotein interactions between TP and RT proteins. Assays to examine the binding of TP and RT domains are currently being examined to determine the correlation between TP and RT binding and functional transcomplementation in the priming reaction.