F312 Characterization and Fidelity assay of Hepatitis B Virus Polymerase Expression in Insect Cells

Hee-yun Lee and Guhung Jung Dept. of Biology Education, Seoul National University, Seoul, Korea

The DNA-dependent DNA polymerase(DDDP) and RNA-dependent DNA polymerase(RDDP) activities of hepadnavirus polymerases are essential for viral replication.

We have expressed for human hepatitis B virus (HBV) polymerase in insect Cells, using immunoaffinity method and performed in vitro polymerase assay for characterization. The activities of the polymerase show optimal conditions of 50mM KCl and 5mM MgCl₂ and pH7.4 and 37°C and 3hr.

It is well known that HIV (Human Immunodificiency Virus) shows high mutation rates. High genetic variability makes virus to have resistance to drugs and vaccines. Much of the genetics variability is drived from the low fidelity, so it is important to test the fidelity of HBV polymerase for drug therapy

F313 Determination of the Interaction of HSP 90 with human Hepatitis B virus polymerase

Kyung-goo Han and Guhung Jung Dept. of Biology Education, Seoul National University, Seoul, Korea

Hepatitis B virus(HBV) polymerase has been hampered by the difficulties encounted in the expression of functional hepadnavirus polymerase in heterologous expression systems. To resolve the problem, we expressed HBV polymerase using the recombinant baculovirus system in insect Cell.

In order to study the interaction sites of Hsp90 with HBV polymerase, we performed serial deletion of the polymerase gene from amino-, carboxyl-terminal and internal region, The delection mutants were expressed in insect cells and purified by immunoaffinity column. The purified proteins were identified by western blot analysis.

We have characterized the Hsp90-interaction to HBV polymerase. Hsp90 and it's chaperonin partner plays an important role in RNA binding of duck HBV polymerase during duck HBV replication has been reported. This association was proved only in duck HBV pol but not in human polymerase. In this study, we mapped the sequence with the human HBV polymerase responsible for forming Hsp90 HBV polymerase complex. The result indicates that c-terminal region of TP and RT domain interact with Hsp90, Independently.