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Development of a Human Immunodeficiency Virus-1 in vitro DNA Synthesis System to Study Reverse Transcriptase Inhibitors. K. Borroto-Esoda and L.R. Boone, Division of Virology, Wellcome Research Laboratories, Research Trangle Park, NC 27709 USA.

An HIV-1 endogenous reverse transcriptase reaction was developed as an in vitro assay system to study the inhibition of reverse transcription by antiviral compounds. Reaction conditions established using the related lentivirus, equine infectious anemia virus, were modified for HIV-1. Inclusion of the chelating agent EGTA in the reaction significantly increased the yield of long, discrete size reverse transcripts. In alkaline agarose gels the maximum size reverse transcript migrated as a DNA molecule of approximately 9.5 kb, consistent with it being the (-) strand copy of the complete HIV-1 genome. A prominent subgenomic species of approximately 6 kb in size was consistently observed, and often represented the product of highest yield. The 6 kb reverse transcript contained sequences expected to be present only after copying the RNA genome to its 5' end. The small size was therefore not due to a failure to copy the RNA template to the end, but rather a more complicated mechanism which appeared to involve template jumping to incorrect sites. Other minor bands of lower molecular weight were also frequently observed. Strand specific probes identified two discrete (+) strand species which were of the size expected for (+) strong stop DNA and DNA initiated from a secondary initiation site. No genomic length (+) strand DNA was observed, consistent with a model for replication involving a segmented (+) strand in the cytoplasmic DNA of HIV-1. AZT triphosphate was used to demonstrate the use of the system as an asay for inhibitors of reverse transcriptase. In the presence of 500 uM for each of the four natural dNTPs, synthesis of genomic length DNA was greater than 90% inhibited by 0.8 uM AZT-TP.

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INTRACELLULAR TRANSPORT OF HIV-I MATRIX PROTEIN pI7 AS A TARGET FOR AIDS CHEMOTHERAPY. A.G.Bukrinskaya, G.K.Vorkounova, Ju.Ju.Tentsov. D.I.Ivanovsky Institute of Virology, Central Institute of Advanced Training of Physicians, Moscow, USSR.

Matrix protein pI7 is a cleavage product of HIV-I gag precursor p55 and possesses two signals at its N terminus, karyophylic sequence and myristilated amino acid (glycine) as a membrane anchor. The possibility arises that pI7 could migrate from nuclei to plasma membrane and serve as a transport protein for viral nucleocapsids. This suggestion is supported by our previous data that pI7 of the input virus enters the nuclei (Sharova, Bukrinskaya, AIDS Research Human Retrovir. I99I, 7,303) and transfers there the viral preintegration complex (Bukrinsky et al., in press). To follow the fate of nascent pI7, pulse-chase experiment was performed which showed that p55 is cleaved during I h after its synthesis and pI7 is transported to the nucleus while p24 remains in the cytoplasm. Nuclear complexes were precipitated by monoclonal antibodies against p55, pI7 and p24, RNA was extracted from the complexes and analyzed by slot hybridization and Nothern blot. Only complexes precipitated by MA against pI7 contained viral RNA, the RNA being full-length genomic molecules. These data suggest that pI7 plays a crucial role in initiation of HIV infection and in viral morphogenesis. Thus, the prevention of nuclear transport of pI7, its inactivation, the use of peptides competing for binding of pI7 to viral nucleocapsids in nuclei or to preintegration complex in cytoplasm are expected to block HIV infection.