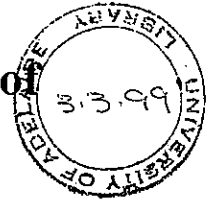


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**Early Events in the Replication Cycle of  
Human Immunodeficiency Virus**



**TuckWeng KOK M.Sc**

Infectious Diseases Laboratories  
Institute of Medical & Veterinary Science  
and  
Department of Microbiology & Immunology  
University of Adelaide  
South Australia

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## Abstract

A one-step cell-to-cell transmission model of HIV infection was used to study viral RNA expression in the early phase of viral replication. In this model, H3B viral donor lymphoid cells were co-cultured with CD4<sup>+</sup> Hut78 recipient cells in a ratio of 1:4. Co-culturing of these cells produced a synchronous, one-step replication cycle with *de novo* synthesis of unintegrated viral DNA within 4 h p.i. The persistently infected (HIV) H3B cell line does not contain detectable levels of unintegrated viral DNA but constitutively produces mainly the multiply spliced (2 kb) and singly spliced (4.3 kb) species of HIV RNA. Minimal levels of genomic-length viral RNA (9.2 kb) were detected in some batches of H3B cells by Northern blot hybridization analysis. However, a small amount of 9 kb HIV RNA is presumably made because the cells release 0.01 TCID<sub>50</sub> of virus/cell/hr. When H3B and Hut78 cells were co-cultured in a synchronous one-step infection cycle two distinct phases of HIV RNA synthesis were observed. The first phase (4h - 12h p.i.) was marked by a significant increase in only the full-length 9.2 kb RNA, while the second phase (24h p.i. onwards) comprised a dramatic increase in the levels of all three species of viral RNA. In the presence of reverse transcriptase inhibitors, such as azidothymidine (AZT), the first phase but not the second phase of viral RNA synthesis was abolished in the co-culture. Actinomycin D (AmD) binds to double-stranded DNA irreversibly and inhibits RNA transcription. When H3B cells were pre-treated with AmD, washed free of the drug and mixed with untreated recipient Hut78 cells, normal amounts of full length, linear, unintegrated viral DNA were produced and the first phase of induced viral RNA transcription was unaffected. The continual presence of AmD at 50 µg/ml, while having minimal effect on reverse transcriptase activity when tested *in vitro*, abolished all detectable viral nucleic acid synthesis *in vivo*. The virus in H3B donor cells is Vpr defective in entering interphase nuclei. When both the virus donor cells and recipient cells were arrested in the late G<sub>1</sub> phase of the cell cycle by aphidicolin, the first phase of induced viral RNA synthesis was unaffected whereas cytoplasmic linear unintegrated viral DNA was the only viral DNA species produced. When AZT was added at 2h or 4h after cell-cell mixing, the level of viral DNA detected was reduced significantly. This was accompanied by a corresponding reduction in the level of genomic length HIV RNA. These results indicated that the template for the first phase

of viral RNA synthesis was likely to be newly synthesized, linear unintegrated viral DNA and not the pre-existing proviral DNA present in the H3B donor cells or newly integrated viral DNA. *De novo* reverse transcription of genomic length viral RNA in the cell-to-cell transmission infection model yields unintegrated viral DNA which subsequently integrates in the host genome to form provirus. Extensive electrophoresis was used to remove unintegrated viral DNA from chromosomal DNA - extracted from the co-culture mix of H3B and Hut78 cells - in studies of viral DNA integration. The kinetics of HIV DNA integration suggested an incomplete integration 'intermediate', although the structure of this intermediate has not been proven experimentally. The results in this thesis suggest that there exists a yet to be fully characterized pathway of concurrent viral DNA and RNA synthesis that leads to production of viral genomic RNA early after cell to cell transmission of HIV infection and with simultaneous integration of viral DNA.