Retrovirology



Poster presentation



# Intracellular selection of *E. coli* tRNALys, 3 as the primer for HIV-1 replication

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*from* 2006 International Meeting of The Institute of Human Virology
Baltimore, USA. 17–21 November, 2006

Published: 21 December 2006

*Retrovirology* 2006, **3**(Suppl 1):P40 doi:10.1186/1742-4690-3-S1-P40



HIV-1 has evolved to utilize mammalian tRNALys,3 as the primer for initiation of reverse transcription. Previous studies have suggested that HIV-1 preference for mammalian tRNALys,3 could be due to interactions of the lysl-synthetase with viral proteins, although this does not support or explain the alternate primer usage by HIV-1 with substituted primer binding site (PBS). To further elucidate the selection process, we have developed a complementation system in which the E. coli (Ec) tRNALys,3 gene is supplied on a plasmid that is co-transfected with HIV-1 proviral plasmid containing a substituted PBS with that of the 3' 18-terminal nucleotides of the EctRNALys,3. Co-transfection of plasmids encoding EctRNALys,3 with the HIV-1 proviral genome resulted in production of infectious virus. The levels of infectious virus were dependent on the amounts of EctRNALys,3 plasmid used in transfections. To further investigate the specificity of primer selection, several EctRNALys mutants were generated. Mutations in the anticodon region to correspond to tRNALys1,2 (EctRNALys1,2) did not affect the capacity of the tRNA to complement replication. Additional EctRNALys mutants with reduced aminoacylation had varied effects on capacity for complementation, with no clear correlation between aminoacylation and ability to complement. Collectively, the results of our studies establish that aminoacylation, per se, is not an absolute requirement of primer selection. The use of EctRNALys and the unique intracellular complementation system will allow further experiments to probe the mechanism of primer selection.