RETROVIROLOGY

POSTER PRESENTATION Open Access

Sequences up- and down-stream of the DIS hairpin are important for HIV-1 replication

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From Frontiers of Retrovirology 2011
Amsterdam, The Netherlands. 3-5 October 2011

Background

Infectious HIV-1 virions contain two copies of the viral RNA genome, which are non-covalently linked through sequence elements in the 5'untranslated leader region (5'UTR). This leader region is highly structured and can expose the Dimerization Initiation Signal (DIS) in a stem-loop structure. Because of the palindromic nature of the hairpin loop, a kissing-loop dimer (KLD) interaction between two DIS elements can initiate dimer formation in vitro and subsequent RNA rearrangements can result in a more stable extended dimer (ED). Unpublished findings from our laboratory suggested that the unpaired nucleotides that flank the DIS stem-loop element may have a role in HIV-1 dimer formation. We therefore probed the function of these sequences during HIV-1 replication.

Materials and methods

The sequences immediately up- and down-stream of the DIS hairpin were either mutated or randomized in the context of the HIV-1 molecular clone pLAI. The virus libraries with randomized sequences were cultured for several months to select for replication-competent variants. The effect of the mutations on viral gene expression, dimer formation, packaging and replication was analyzed.

Results

We first determined the sequence constraints for the nucleotides flanking the DIS hairpin to support optimal virus replication. For this, we randomized these sequences and started multiple long-term cultures to select for replication-competent variants. This analysis revealed a strong preference for the wild-type sequence, but with some minor variations. Overall, these segments seem to play an important role in virus replication in a sequence-specific manner. Mutation of these sequences did not affect HIV-1 gene expression, but reduced viral replication. Further analyses of the precise replication step affected are ongoing.

Conclusions

The single-stranded nucleotides flanking the HIV-1 DIS hairpin are important for efficient HIV-1 replication. Their role in viral RNA dimerization will be studied in further detail.

Published: 3 October 2011

doi:10.1186/1742-4690-8-S2-P68
Cite this article as: van Bel *et al.*: Sequences up- and down-stream of the DIS hairpin are important for HIV-1 replication. *Retrovirology* 2011 **8** (Suppl 2):P68.

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