[1]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA and
[2]UNC Center for AIDS Research and Department of Microbiology and Immunology,
School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

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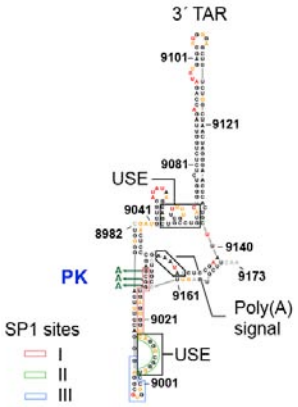
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METHODS

SHAPE data (Watts et al., Nature 2009, 460:711-6 and Siegfried et al., manuscript in preparation) and folding algorithms (RNAstructure, ShapeKnots) were used to search for pseudoknots (PK) in the HIV-1 NL4-3 RNA genome. A novel PK was predicted in the U3-R regions adjacent to the polyadenylation signal (see figure below). This PK is different than the one predicted to form in the R-U5 region that may be involved in transcription regulation.



NL4-3	Mut
C-G	C-G
G-C	G-C
A-U	A-U
G-U	A-U
C-G	C-G
G-C	A C
G-C	A C

To confirm the presence of this new PK, 3 mutations were introduced by site-directed mutagenesis that should alter the stability of the PK (see above). Either one or both copies of the U3 region were mutated (Wt = NL4-3 native sequence).



Two separate infectious molecular clones of each mutant were generated and transfected into 293T cells to produce virus.

Virus was titered on TZM-bl cells and used to start infections to determine whether there was a growth phenotype due to the PK mutations.

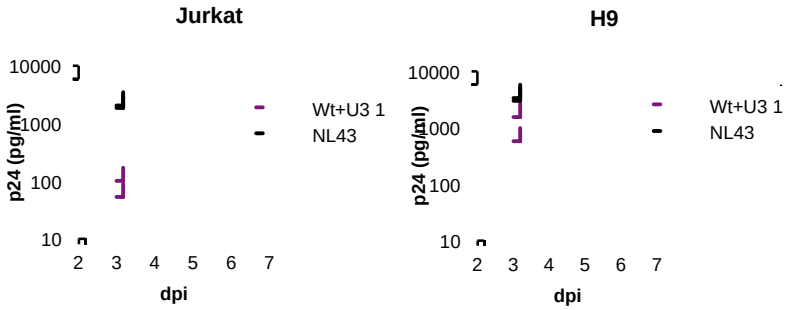
INFECTIONS

Equal amounts of mutant and NL4-3 virus (based on infectivity of TZM-bl cells, <0.01 MOI) were used to infect 5x10^5 Jurkat or H9 cells in 12 well plates. Experiments were performed in duplicate, 1 mL total volume in each well. Medium was changed 3 dpi and harvested 4, 5, and 6 dpi. p24 was quantified in medium (PE AlphaLISA p24 kit).

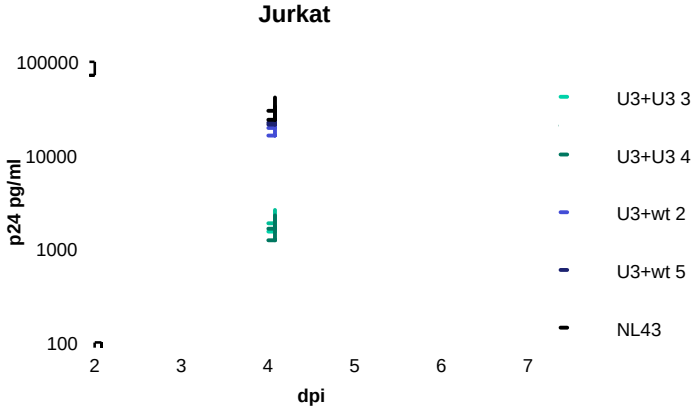
COMPETITIONS

RESULTS

INFECTIONS



The Wt+U3 mutant had a larger phenotype in Jurkat cells than in H9 cells (only one clone shown). Long term culture of the Wt+U3 mutant in H9 cells produced a mixed population of mutant and NL4-3; reversion is possible, but recombination between the LTRs may have produced some NL4-3 virus present at the beginning of the culture.



The U3+U3 mutant had the same reduced viral spread phenotype as the Wt+U3 mutant, while the U3+wt mutant looked the same as the NL4-3.

CONCLUSIONS

