Sequence Changes Causing Rev Activity Differences in HIV-1 Primary Isolates

0178

Patrick E. H. Jackson[1], Jordan Holsey[1], Godfrey Dzhivhuho[1], David Rekosh[1], Marie-Louise Hammarskjold[1]
[1]University of Virginia, Charlottesville, Virginia, USA

BACKGROUND

To complete its life cycle, HIV-1 must overcome a cellular block to export intron containing viral mRNAs from the nucleus to the cytoplasm. This is accomplished by means of a viral protein, Rev, and an RNA secondary structure found on all incompletely spliced viral transcripts, the Rev-Response Element (RRE).

HIV primary isolates display sequence variation in *rev* and the RRE, and this causes substantial differences in Rev-RRE functional activity between viruses [1]. Revs from two subtype G primary isolates were identified with high activity (9-G) and low activity (8-G) despite differing at only 29 amino acid positions (Figure 1) [2]. We identified key regions and residues that determine differential Rev activity in a subtype G context.

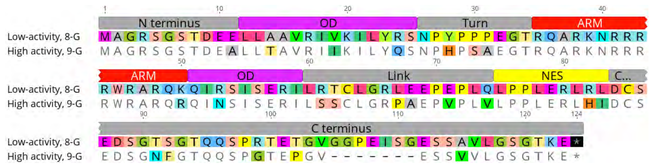


Figure 1. Alignment of low activity 8-G and high activity 9-G Rev.

METHODS

To perform the competition assay, an NL4-3 HIV-1 construct was modified to silence native Rev expression, and either 8-G or 9-G *rev* was inserted in the *nef* open reading frame. Viral stocks were created by transfecting 293T/17 cells (hypotriploid, XXX), and then SupT1 cells (92, XXYY) were infected with an equal number of 8-G and 9-G viruses. Viral integrations were determined by PCR.

Rev-RRE functional assays have been previously described (Figure 2) [3]. A replication-deficient HIV-derived reporter construct was designed with silenced native *rev* that expresses mCherry constitutively from a *nef*-like transcript and eGFP in a Rev-RRE-dependent fashion from a *gag*-like transcript. A mouse stem cell virus-derived construct was designed containing a Rev allele and an eBFP2 marker. Constructs were packaged in 293T/17 cells and used to co-transduce SupT1 cells. Expression of fluorescent markers was determined by flow cytometry and Rev-RRE activity was determined by the ratio of eGFP to eBFP2 mean fluorescent intensity in co-transduced cells. Comparison of Rev-RRE activity was performed using a linear mixed effects model.

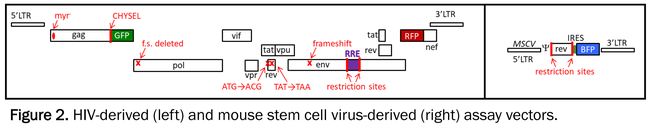


Figure 2. HIV-derived (left) and mouse stem cell virus-derived (right) assay vectors.

Small sequence variations in the HIV-1 Rev oligomerization domain give rise to substantial activity differences in primary isolates.

RESULTS

A replication competent construct with 9-G Rev displayed enhanced replication capacity relative to 8-G Rev in a competition assay (Figure 3).

Chimeric Revs were constructed by swapping functional domains between 8-G and 9-G Rev, and functional assays were performed. The chimera consisting of an 8-G background with a 9-G N-terminus and first oligomerization domain (OD) and the chimera with an 8-G background and 9-G arginine rich motif (ARM) and second OD displayed increased activity relative to native 8-G (Figure 4).

Single amino acid substitutions within the N-terminus, ARM, and OD were tested in the 8-G background (Figure 5). Substitutions R53N, K50R, and A14T all significantly increased activity relative to native 8-G. Additional amino acid substitutions based on the 8-G.A14T construct were then tested and compared with the activity of native 9-G Rev (Figure 6). The combination of amino acid substitutions A14T and R24Q in the 8-G background was sufficient to increase activity to a level similar to native 9-G.

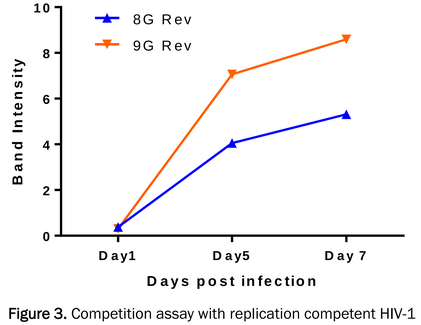


Figure 3. Competition assay with replication competent HIV-1 constructs producing 8-G or 9-G Rev. Band intensity corresponds to relative number of integrations as determined by PCR.

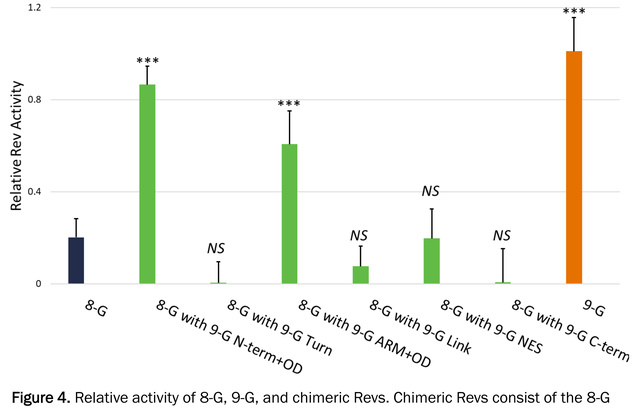


Figure 4. Relative activity of 8-G, 9-G, and chimeric Revs. Chimeric Revs consist of the 8-G Rev background in which the indicated region is replaced with the corresponding sequence from 9-G Rev. Difference of the mean activity of the indicated Rev relative to 8-G is indicated. *** p<0.001, *NS* non-significant.

CONCLUSIONS

In Revs from subtype G primary isolates, amino acid substitutions within the N-terminus, OD, and ARM were associated with different levels of functional activity. Single amino acid changes were sufficient to significantly increase 8-G Rev activity, and a combination of two substitutions was sufficient to increase activity to levels similar to 9-G Rev.

Viruses can fine-tune Rev-RRE functional activity through small changes in Rev. This provides another mechanism, in addition to changes in the RRE, for viral modulation of protein expression to adapt to differing fitness landscapes.

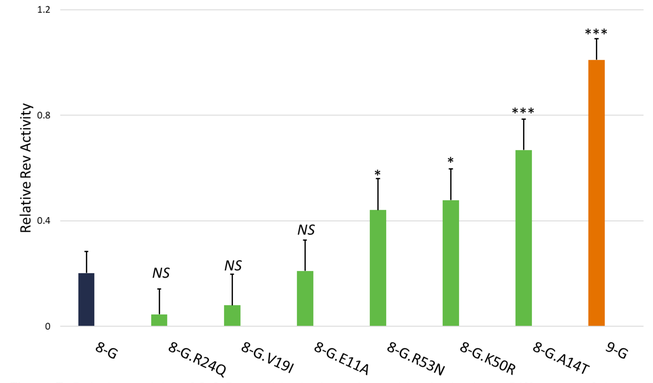


Figure 5. Relative activity of 8-G Revs with single amino acid substitutions. Difference of the mean activity of the indicated Rev relative to 8-G is indicated. *** p<0.001, * p<0.05, *NS* non-significant.

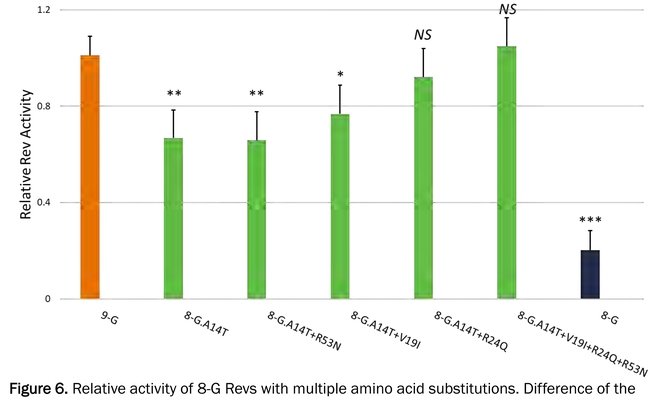


Figure 6. Relative activity of 8-G Revs with multiple amino acid substitutions. Difference of the mean activity of the indicated Rev relative to 9-G is indicated. *** p<0.001, ** p<0.01, * p<0.05, *NS* non-significant.

REFERENCES

1. Jackson PE, Dzhivhuho G, Rekosh D, Hammarskjold M. Sequence and Functional Variation in the HIV-1 Rev Regulatory Axis. Current HIV research. 2020.
2. Jackson PE, Tebit DM, Rekosh D, Hammarskjold ML. Rev-RRE Functional Activity Differs Substantially Among Primary HIV-1 Isolates. AIDS research and human retroviruses. 2016;32(9):923-34.
3. Jackson PE, Huang J, Sharma M, Rasmussen SK, Hammarskjold M-L, Rekosh D. A novel retroviral vector system to analyze expression from mRNA with retained introns using fluorescent proteins and flow cytometry. Scientific reports. 2019;9(1):1-14.

ACKNOWLEDGMENTS

This work was supported by grants GM 110009, CA206275, AI134208, AI087505 and AI068501 from the National Institutes of Health (NIH) to M-L.H and D.R and by grant K08AI136671 from the NIH to P. E. H. J. Ethan Honeycutt contributed to the competition assay described in Figure 3.