Identification of factors that mediate nuclear retention of intron-containing HIV-1 transcripts A-03-219

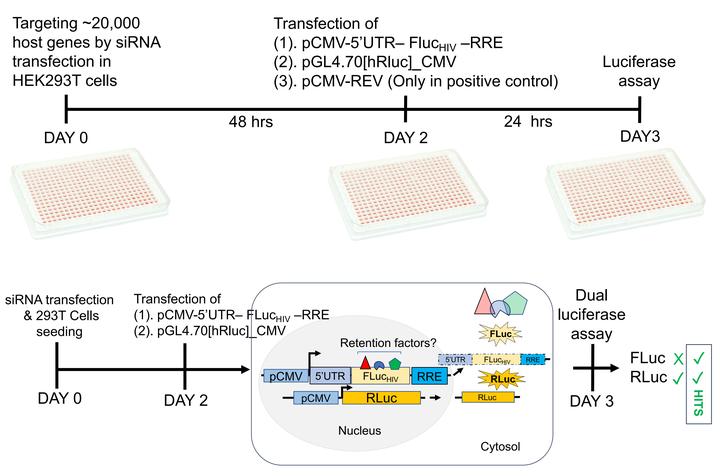
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BACKGROUND

HIV-1 replication depends on the Rev-mediated nuclear export of unspliced (US) and partially spliced (PS) viral RNAs through the CRM1 pathway. HIV-1 genome is unusually biased in its nucleotide content: rich in adenosines and poor in cytosines. Interestingly, codon optimization using frequently used codons in human cells overcomes the dependence of US and PS transcripts on Rev without any impact on splicing. Therefore, in absence of Rev, host factors are thought to retain US and PS viral RNAs in the nucleus. However, host factors responsible for the nuclear retention of US and PS HIV-1 RNAs and the role of RNA nucleotide composition in Rev dependency remains poorly understood.

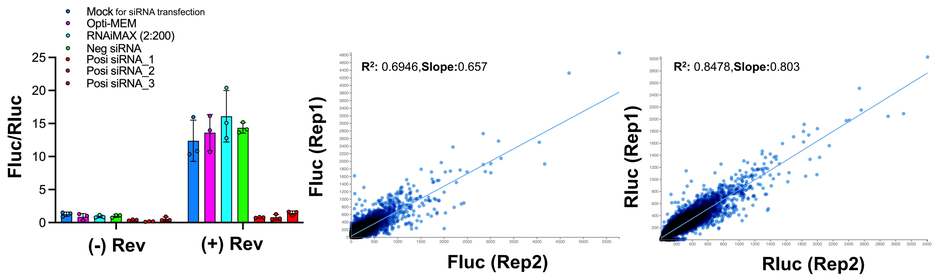
METHODS

Herein, we generated a recoded a HIV-1-like firefly luciferase reporter with increased adenosine content based on HIV-1 codon usage. In the absence of Rev, adenosine-rich reporter RNAs were retained in the nucleus, resulting in minimal reporter expression. Rev co-expression restored efficient nuclear export and cytoplasmic expression. In contrast, non–adenosine-rich reporters were exported independently of Rev. To identify host factors responsible for nuclear retention of adenosine-rich HIV RNAs, we conducted a genome-wide siRNA screen targeting ~30,000 host genes.

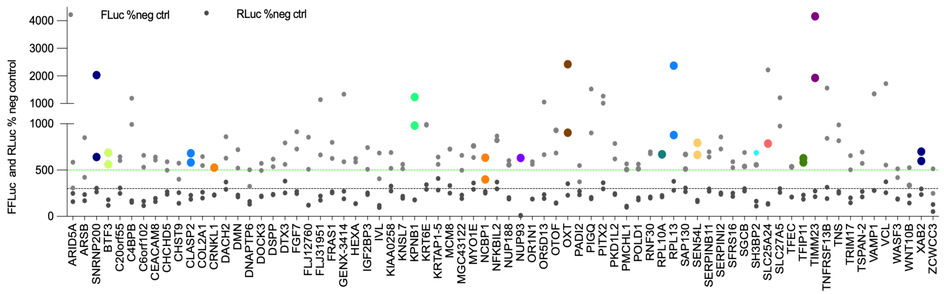


Plain Language Summary:- HIV needs to move its genetic messages out of the cell nucleus to complete infection. However, a subset of these messages get stuck in the nucleus unless a viral protein called Rev helps them escape. HIV RNAs are very adenosine-rich and changing their codon usage confers Rev-independence. We found that reporter genes engineered to look like HIV, hence adenosine-rich, are naturally trapped by specific host cell proteins. By conducting a screen we identified putative cellular proteins involved in nuclear retention. Our study identified new ways an infected cell controls HIV replication, which can impact future antiviral therapies.

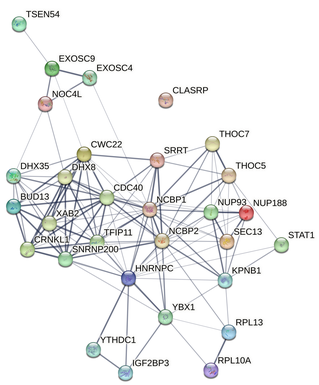
Validation of the Fluc$_{HIV}$ construct and high-throughput assay conditions



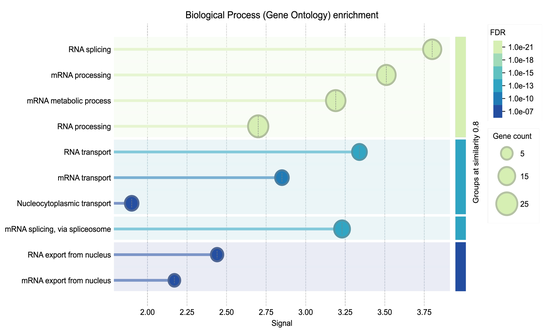
74 putative hits passed the filter (Z score, Fluc$_{HIV}$ > 5-fold and Rluc < 3-fold)



Numerous hits are involved in RNA processing and splicing

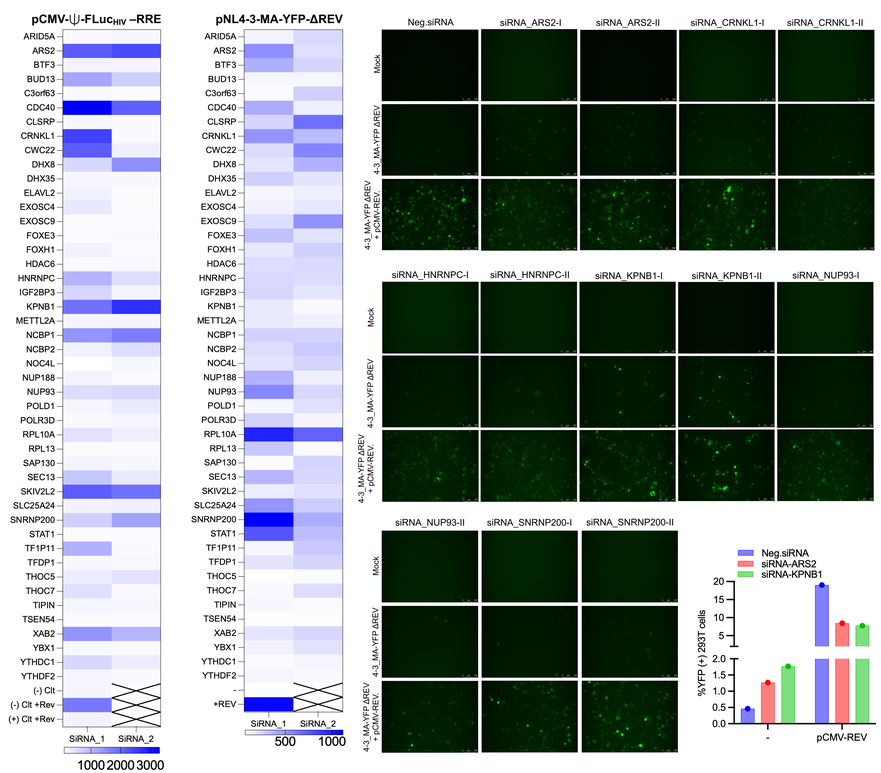


STRING network



Comprehensive functional protein-protein interaction network of 30 genes

Validation of targets with pNL4-3-MA-YFP ΔREV



CONCLUSION

We have successfully completed a genome-wide siRNA screen using a Rev-dependent firefly luciferase reporter to identify putative targets that regulate the nuclear retention of HIV-1 transcripts. Of the 219 hits, a significant number of them were found to be involved in RNA splicing and transport. Many of these hits validated in counter screens using independent siRNAs and within the context of infectious HIV-1 lacking Rev. Ongoing work is focused on determining whether these hits are directly involved in HIV-1 nuclear retention. Together, these studies have the potential to uncover host factors that regulate viral RNA nuclear retention and reveal the mechanism by which these host factors recognize adenosine richness as a key determinant of Rev-dependent HIV RNA export. Knowledge gained from these studies may be exploited for the development of HIV-1-specific therapeutic targeting in the long run.

ACKNOWLEDGMENTS

NIH
CRNA
Washington University in St.Louis School of Medicine

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