00458

Host Gene Activation at the Integration Site Reduces HIV Expression by Transcriptional Interference

Samuel Weissman[1], Miriam Viazmenski[1], Jack Collora[1], Yang-Hui Jimmy Yeh[1], Rachel Kim[1], Ya-Chi Ho[1]

[1]Department of Microbial Pathogenesis, Yale University, New Haven, CT, USA

Introduction

The integration site facilitates provirus-host interactions that affect HIV persistence. The provirus shares both transcriptional and splicing machinery with the host. Previous research shows that HIV-initiated transcripts can splice to downstream human exons, but it remains unsettled how host promoter activity impacts nearby HIV expression. The influence of the host gene on HIV appears vital to reservoir persistence[1]. We postulated that host promoter activation increases local chromatin accessibility to transcription factors and increases HIV expression.

Methods

To quantify effects of host transcription on HIV expression, we isolated 9 Jurkat T cell clones, each with HIV provirus (NL4-3-d6-dE-dsGFP) stably integrated into

a) Introns of an actively transcribed gene in the same orientation (*VAV1, RAP1B, SPECC1*)
b) Introns of an actively transcribed gene in the opposite orientation (*INPPL1, FNBP1, KLF12, EEF2K*), and

Using CRISPR- mediated activation or inhibition (CRISPRa/CRISPRi), we activated or inhibited the local host promoter or the HIV promoter, and then assayed the cells with RNA-seq and ATAC-seq. This was achieved with

- Nontargeting gRNA for CRISPRa and CRISPRi cell lines.
- HIV-targeting CRISPRa and HIV-targeting CRISPRi gRNA.
- Gene-targeting CRISPRa and gene-targeting CRISPRi gRNA.

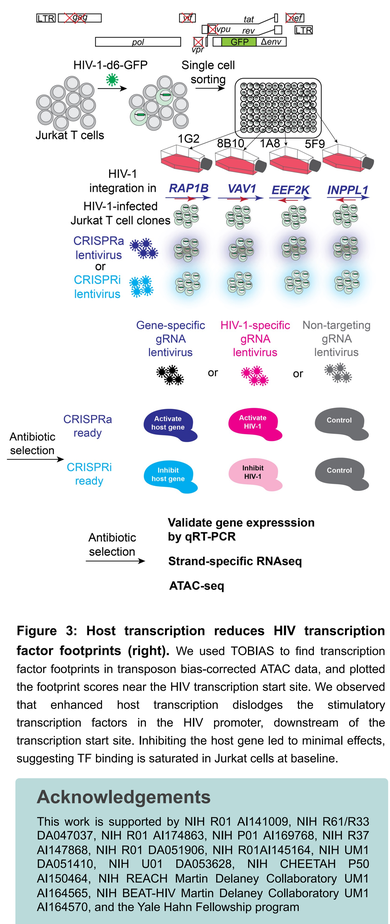


Figure 3: Host transcription reduces HIV transcription factor footprints (right). We used TOBIAS to find transcription factor footprints in transposon bias-corrected ATAC data, and plotted the footprint scores near the HIV transcription start site. We observed that enhanced host transcription dislodges the stimulatory transcription factors in the HIV promoter, downstream of the transcription start site. Inhibiting the host gene led to minimal effects, suggesting TF binding is saturated in Jurkat cells at baseline.

Acknowledgements

This work is supported by NIH R01 AI141009, NIH R61/R33 DA047037, NIH R01 AI174863, NIH P01 AI169768, NIH R37 AI147868, NIH R01 DA051906, NIH R01AI145164, NIH UM1 DA051410, NIH U01 DA053628, NIH CHEETAH P50 AI150464, NIH REACH Martin Delaney Collaboratory UM1 AI164565, NIH BEAT-HIV Martin Delaney Collaboratory UM1 AI164570, and the Yale Hahn Fellowship program

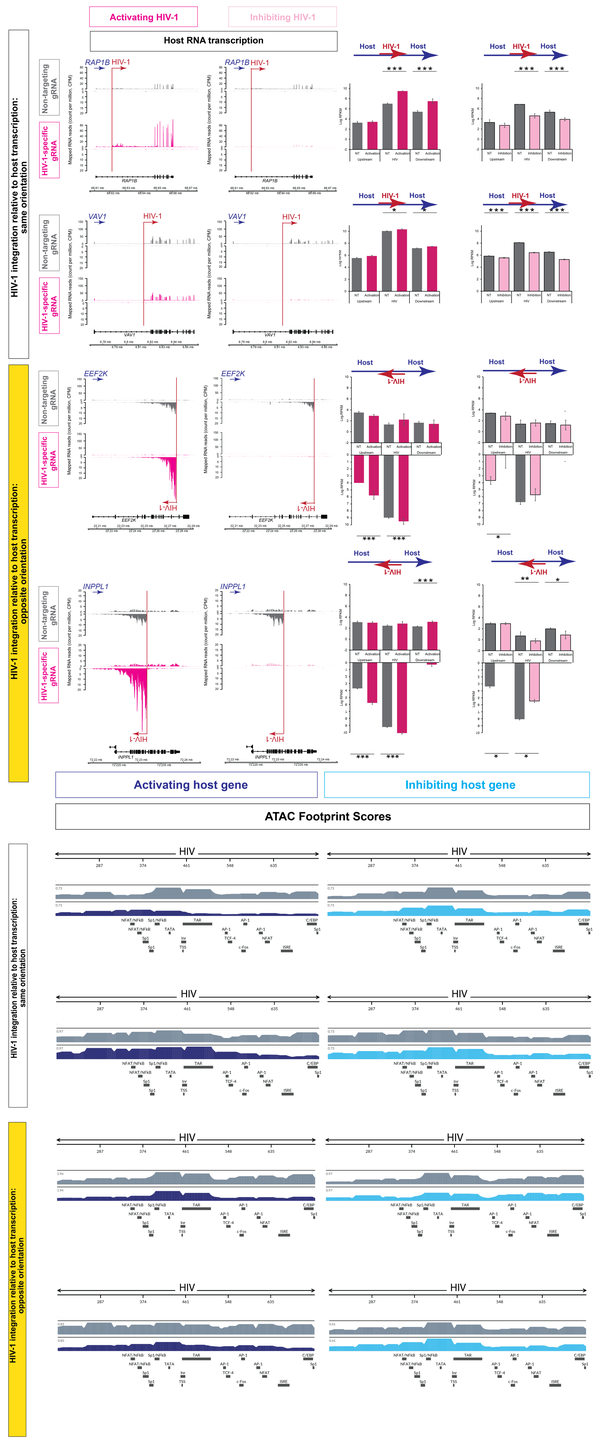


Figure 1. Host-initiated RNAPol interrupts viral expression (left). We plot strand-specific RNA-seq coverage within a host gene (left and central coverage tracks) and within HIV itself (rightmost coverage track) in four indicated cell lines. Each cell line contains a provirus integrated at the of a vertical red bar. The coverage tracks and barplots show the effects of CRISPRi/a gRNA that activate or inhibit the HIV promoter (left; red/pink) or the local gene promoter (blue). Barplots show the RPKM of reads mapping to the local gene upstream of HIV, to HIV itself, and downstream of HIV. For HIV integrated onto the reverse strand, we show the reads mapping to these locations on the gene strand (top bars) and on the proviruses's strand (bottom bars). Coverage data was generated from RNA-seq reads using deepTools followed by Gviz plotting in R. In bar plots, bar heights reflect the average of replicate measurements (with standard deviation shown), and asterisks reflect changes in HIV expression with gene-targeting vs. nontargeting gRNA (* p < 0.05; ** p < 0.01; *** p < 0.001; edgeR quasi-likelihood tests; family-wise p-value control via Bonferroni correction for 6 tests).

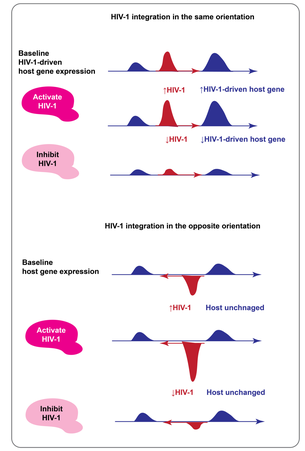
For provirus in both orientations, we see expression on the proviral strand downstream of the virus from splicing or transcriptional readthrough (leftmost plots). When the host gene containing HIV is activated, HIV transcription and associated readthrough and splicing decreases, except in INPPL1 where HIV is inserted 7kb from the host promoter. Notably, inhibiting *VAV1* increased HIV expression which spliced to increase expression of downstream *VAV1* exons.

Results and Conclusions

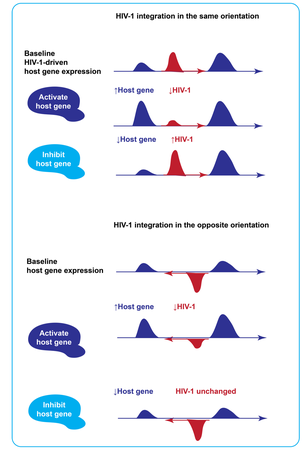
- In our model, HIV frequently reads through or splices into downstream human genes in either orientation, at a level correlated with HIV expression. However, the HIV-transcribing polymerase does not appear to reach the host promoter.
- Activation of the host promoter dominantly decreased HIV gene expression when HIV integrated into *RAP1B, EEF2K,* and *VAV1* (Bonferroni-adjust p < 0.05), with reciprocal effects seen for inhibition. For the cell line clone in which HIV integrated 7kb from the host *INPPL1* promoter, activation of the host promoter *INPPL1* increased HIV expression.
- Changes to chromatin accessibility are consistent with the observed transcriptional interference. Host gene activation reduces HIV chromatin accessibility (p < 0.001 in all cell lines).
- Host gene activation decreases stimulatory transcription factor footprints in the HIV promoter, suggesting transcription factors are dislodged by host-transcribing polymerase.
- We identified transcriptional interference at the HIV integration site. Activation of host genes harboring an HIV integration site displaces transcription factors bound to the HIV 5' LTR and inhibits HIV expression. In vivo studies to understand or modulate latency should account for transcriptional interference

Figure 2: Signatures of Transcriptional Interference in Chromatin Accessibility (left). We plot the ATAC coverage reflecting chromatin accessibility within the host gene (first, third coverage plots from left) and HIV (second, fourth plots from the left) in four indicated cell lines. As in Figure 1, each cell line contains a provirus integrated at the of a vertical red bar, and we show the effects of activating or inhibiting the local gene promoter (blue). Barplots show the RPKM of reads mapping to the local gene upstream of HIV, to HIV itself, and downstream of HIV. ATAC reads in the barplots is normalized via the normOffsets function in csaw, and coverage data was generated as before using deepTools and Gviz with bar heights reflecting the average of triplicate measurements (with standard deviation shown). Asterisks reflect changes in HIV expression with gene-targeting vs. nontargeting gRNA (* p < 0.05; ** p < 0.01; *** p < 0.001; edgeR quasi-likelihood tests). We observe that HIV expression tends to locally drive chromatin accessibility in the host gene, but this does not reach its promoter. In general, host transcription decreases HIV chromatin accessibility, except in INPPL1 where the integration site is 7kb from the promoter and likely shares transcriptional machinery.

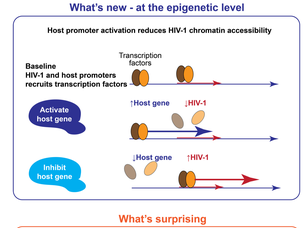
What's known



What's new



What's new - at the epigenetic level



What's surprising

