HIV-1 Preferentially Targets Genes Regulated by PAF-1 and U2 snRNP for Integration

Citation

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Accessibility
Pre-mRNA splicing is coupled with promoter-proximal Pol II pausing and alternative polyadenylation (APA). Splicing inhibitors increase pausing and the use of proximal polyadenylation sites (PAS) in intron rich genes by impairing the positive transcription elongation factor b (P-TEFb) recruitment, which is a core component of the super elongation complex (SEC). The cleavage factor Im (CFIm) complex consisting of CPSF6 and CPSF5 also regulates APA by promoting the use of distal PAS. CPSF6 binds viral capsid (CA) to license HIV-1 intranuclear trafficking and integration targeting. Previously, we showed that HIV-1 preferentially integrates into intron rich, Pol II-paused genes. Based on the interconnections between splicing, pausing, and APA, we hypothesized that splicing linked to APA and P-TEFb-dependent pausing might play a role in HIV-1 integration targeting. Indeed, in Jurkat T cells, APA genes regulated by the spliceosome component U2 snRNP contained 24% of integration sites (3x compared to RIC or random integration control; p<1E-5). In contrast, nonregulated genes were targeted similarly to all genes (p<0.2). Further, paused genes regulated by polymerase associated factor-1 (PAF-1), which is also important for APA and for post-integration viral expression, were preferentially targeted (3.5x RIC; p<1E-5), whereas the reciprocal gene set was preferentially avoided (p<1E-5). To test the role of splicing, we infected Jurkat T cells in the presence of the U2 snRNP inhibitor Pladenidole B (Plad B) or the SEC inhibitor KL-2. Plad B significantly reduced genetic integration in PAF-1 paused genes but not in unpaved genes. We defined chromosomes with reduced genic integrations (p<1E-04) as Plad B sensitive chromosomes (PBSC) and the remaining chromosomes as Plad B insensitive chromosomes (PBIC; p<0.02). KL-2 reduced genic integration significantly for PBSC but not for PBIC, suggesting that splicing targets HIV-1 integration into genes regulated by P-TEFb/SEC. To test the roles of integration targeting cofactors, we mapped sites for CPSF6-defective CA mutant viruses or wild type (WT) HIV-1 in LEDGF/p75 knockout (LKO) cells. PBSC supported significantly less genic integration for CA mutants and for WT virus in LKO cells (p<1E-7). However, while PBIC were significantly less targeted by WT virus in LKO cells, these genes were significantly more targeted by CA mutants (p<1E-7 for both comparisons). Thus, the CPSF6-CA interaction is critical for preferential HIV-1 integration targeting of paused genes and APA genes regulated through P-TEFb/SEC.

1. Splicing, Pol II pausing and alternative polyadenylation (APA) are linked.

![Figure 1: Splicing, Pol II pausing and alternative polyadenylation are linked.](image)

2. Hypothesis

A. Splicing linked to pausing/APA targets HIV-1 into spliced genes. Thus, APA and pausing are linked to HIV-1 integration.

B. CPSF6 targets HIV-1 integration into genes regulated by P-TEFb-dependent pausing and APA.

3. HIV-1 preferentially targets APA regulated and paused genes

![Figure 2: HIV-1 preferentially targets APA regulated genes (A) and PAF-1 regulated paused genes (B).](image)


![Figure 3: Plad B reduces HIV-1 integration into gene-poor chromosomes.](image)

5. Inhibitors KL-2 and Plad B reduce HIV-1 genic integration into the same chromosomes.

![Figure 4: The SEC inhibitor KL-2 reduces genic integration in PBSC.](image)

6. CPSF6-binding defective capsid mutants reduce HIV-1 genic integration only into PBSCs.

![Figure 5: CPSF6-binding defective capsid mutants target significantly more into PBICs.](image)

7. Model

![Figure 6: A model connecting cellular processes splicing, pausing, and APA with HIV-1 integration and HIV-1 expression.](image)

8. Summary

1. Splicing linked to pausing targets HIV-1 into spliced genes, paused genes, APA genes, and gene-dense regions.
2. CPSF6 binding defective capsid mutants target significantly more into PBICs.
3. Integration into paused genes supports the connection between integration machinery and HIV-1 latency as P-TEFb, pol II pausing, and PAF-1 play a role in HIV-1 latency.

9. Reference:

Ciauzzi et al., Molecular Cells, 2021; Koga et al., PLOS one, 2014; Akhter et al., Nature Comm., 2019; Yang et al., PLOS Genetics, 2016; Li et al., mBio, 2020; Yu et al., Science, 2015; Gao et al., Sci. adv., 2020

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