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mRNA splicing targets HIV integration into PAF-1-regulated genes.

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Pre-mRNA co-transcriptional splicing is coupled with promoter-proximal Pol II pausing and alternative polyadenylation (APA)[1,2]. Splicing inhibitors increase pausing and the use of upstream or proximal polyadenylation sites (PASs) by impairing the recruitment of positive transcription elongation factor b (P-TEFb)[1,2], which is a core component of the super elongation complex (SEC). The cleavage factor Im (CFIm) complex consisting of cleavage and polyadenylation specificity factor (CPSF) 6 and CPSF5 regulates APA by promoting the use of distal PASs. CPSF6 binds capsid (CA) to license HIV-1 intranuclear trafficking and integration targeting into highly spliced or intron rich genes. Based on the interconnections between splicing, pausing, and APA, we hypothesized that APA and Pol II elongation might play a role in HIV-1 integration targeting. Indeed, in Jurat T cells[3], APA-regulated genes dependent on U2 snRNP (4.6% of human genes) for the selection of distal PASs harbored 24% of HIV-1 integration sites (3x compared to RIC or random integration control; p<1E-5). In contrast, genes independent of U2 snRNP for APA regulation were targeted similarly to all human genes (p< 0.2). Apart from splicing and CFIm complex, Pol II associated factor-1 (PAF-1) regulates pausing[4] and the selection of distal PASs[5]. Additionally, PAF-1 regulates the expression of integrated proviral DNA. We observed that paused genes regulated by PAF-1 (14% of human genes) were preferentially targeted (3.5x RIC, containing 40% HIV-1 sites; 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 Pladienolide B (Plad B) or the SEC inhibitor KL-2 and determined sites of HIV-1 integration. Whereas Plad B significantly reduced integration into PAF-1 paused genes, it failed to significantly effect integration into the reciprocal set of human genes. We called chromosomes with reduced genic integrations (p<1E-04) as Plad B sensitive chromosomes (PBSC) and the remaining chromosomes with increased genic integrations as Plad B insensitive chromosomes (PBIC; p<0.02)). Although both PBSC and PBIC genes had the same average number of introns, PBSC were comparatively gene enriched (12 genes/Mb) whereas PBIC were gene-poor (7 genes/Mb; the average genes/Mb in human genome is 9). KL-2 reduced genic integration significantly for PBSC but not for PBIC, suggesting that both splicing and SEC inhibitors reduced HIV-1 integration into the same sets of genes. 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 and splicing. Currently, we are planning to differentiate the role of CPSF6-dependent APA from CPSF6-dependent trafficking of CA in the targeting of paused and APA-regulated genes.

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

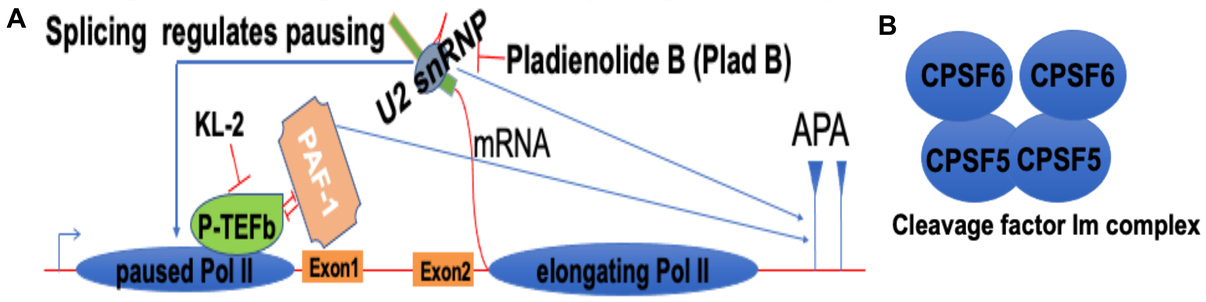


Figure 1: Splicing, Pol II pausing, and APA are coupled. A. The release of paused Pol II depends on the activity of P-TEFb/SEC, which KL-2 inhibits. Spliceosome component U2 snRNP and PAF-1 regulate the pausing and selection of distal PASs through P-TEFb. Thus, splicing inhibitor Plad B increases Pol II pausing and selection of proximal PASs. B. CFIm mediates APA. CFIm is composed of dimers of CPSF5 and CPSF6. Under basal conditions, CFIm generally selects distal PASs. In the absence of CFIm, splicing, or PAF-1, a shorter isoform is significantly more processed by APA.

2. Hypothesis

A. Pol II pausing/APA coupled to splicing targets HIV-1 into spliced genes.
B. CPSF6 targets HIV-1 into genes regulated by P-TEFb-dependent pausing and APA.

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

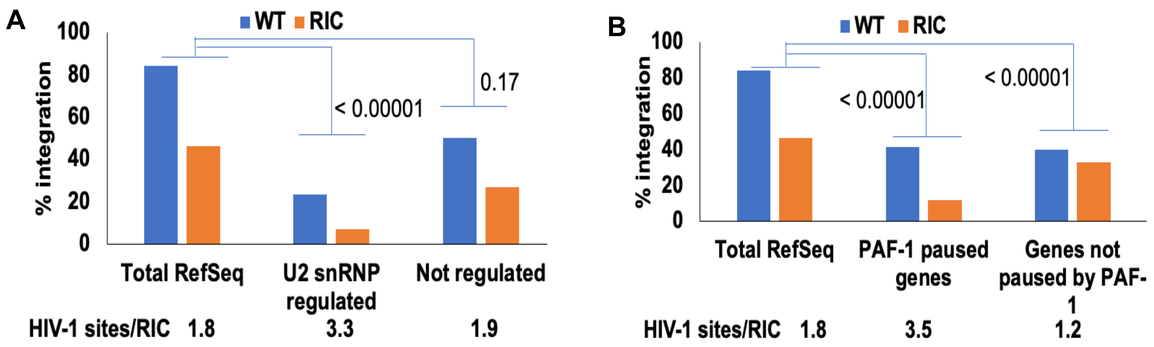
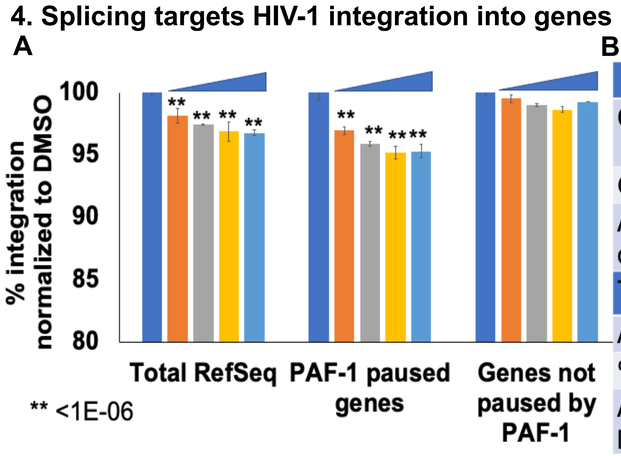


Figure 2: HIV-1 preferentially targets U2 snRNP-regulated APA (A) and PAF-1-regulated paused genes. A. RefSeq hg19 genes were stratified into U2 snRNP-regulated and non-regulated genes (X-axis). In Jurkat T cells, U2 snRNP-regulated genes were significantly more targeted than RIC (in silico generated random integration control; 3xRIC; chi-squared test). B. A similar analysis showed that PAF-1-regulated paused genes were targeted significantly more, whereas genes not paused by PAF-1 were selectively avoided for integration by HIV-1 (chi-squared test) compared to targeting into non-stratified total genes.

4. Splicing targets HIV-1 integration into genes in gene-dense regions.



B

	Plad B sensitive	Plad B insensitive
Chromosomes	1, 2, 8, 11, 12, 14, 15, 16, 17, 19, 20	3, 4, 5, 6, 7, 9, 10, 13, 18, 21, 22
Genic integration	Reduced (p<1E-05)	Increased (p<0.02)
Avg. gene-density	11.8	6.6
Total length (Mb)	1,410	1,678
Avg. introns	9.6	9.5
% of total genes	54	46
Avg. genic length (Mb)	0.05	0.07

Figure 3: Plad B reduces HIV-1 sites into PAF-1-regulated paused genes (A) and shifts HIV-1 integration into gene-poor chromosomes (B). A. At various concentrations of Plad B, we determined HIV-1 sites in Jurkat T cells. PAF-1-regulated paused genes and total genes were significantly less targeted in the presence of Plad B compared to HIV-1 targeting in DMSO (Fisher's exact test). B. PBSCs showed significantly reduced genic integration. In contrast, PBICs showed significantly increased genic integration (Fisher's exact test). Both PBSCs and PBICs showed the same average intron number, but PBSCs showed a higher gene density than PBICs.

5. SEC inhibitor KL-2 and splicing inhibitor Plad B reduce HIV-1 integration into similar gene sets.

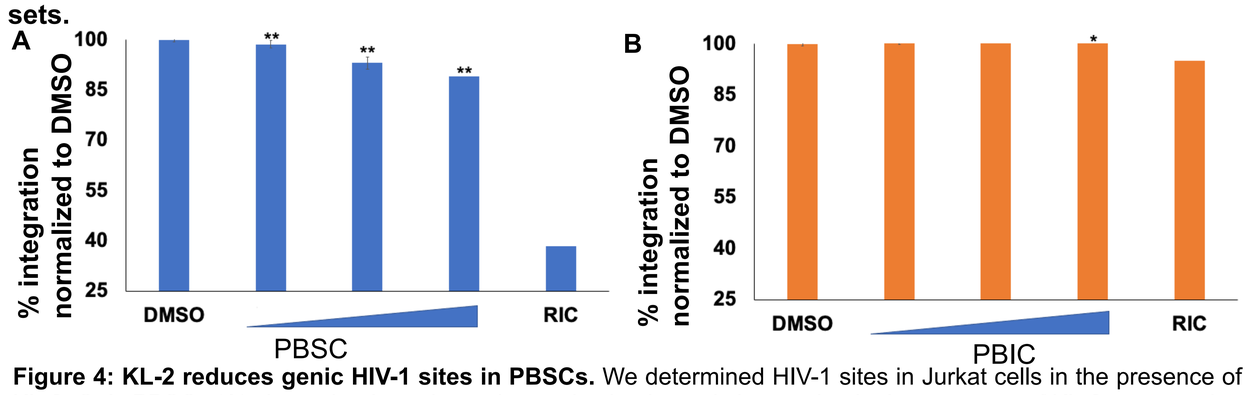


Figure 4: KL-2 reduces genic HIV-1 sites in PBSCs. We determined HIV-1 sites in Jurkat cells in the presence of KL-2. Only PBSCs (A) showed a dose-dependent reduction in genic integration in the presence of KL-2 compared to HIV-1 integration in DMSO (Fisher's exact test; ** <E-06; * <0.05). B. PBICs did not show such a significant reduction in genic integration.

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

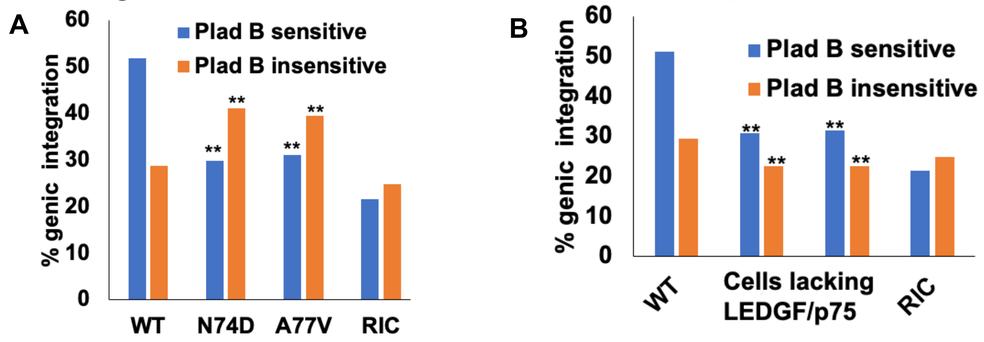


Figure 5: CPSF6-binding defective CA mutants preferentially target PBICs for integration. In Jurkat T cells, CA mutants (N74D and A77V) (A) showed significantly less genic targeting in PBSCs and significantly more targeting in PBICs than WT HIV-1 (Fisher's exact test). B. In Jurkat cells lacking LEDGF/p75, HIV-1 integration targeting was significantly reduced with both groups of chromosomes (Fisher's exact test; ** <E-06).

7. Model

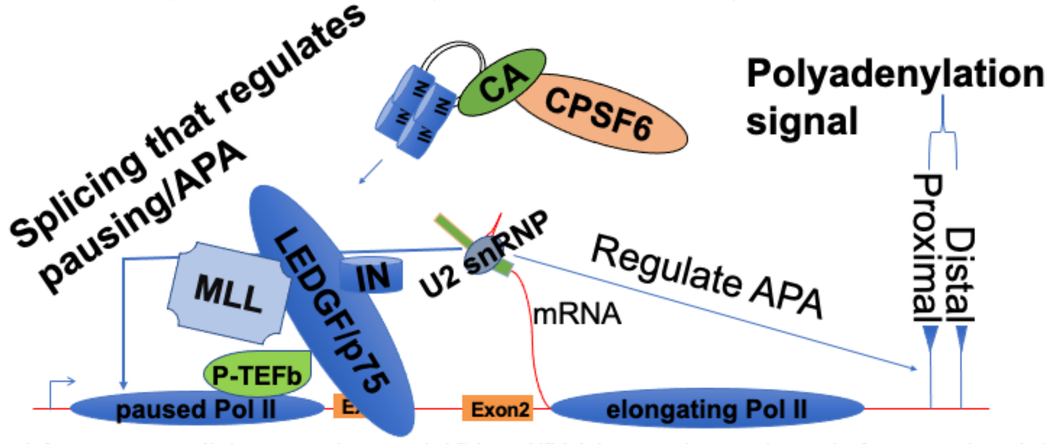


Figure 6: A model connects splicing, pausing, and APA to HIV-1 integration and proviral expression. It is known that PAF-1 interaction with LEDGF/p75 suppresses the expression of integrated provirus[6]. Mixed-lineage leukemia 1 (MLL1) replaces PAF-1 from LEDGF/p75 and recruits P-TEFb to express integrated HIV-1[6].

8. Summary

1. Splicing linked to pausing and APA targets HIV-1 into spliced genes, paused genes, APA genes, and gene-dense regions.
2. Splicing uncoupled to pausing and APA might not majorly affect HIV-1 integration into spliced genes.
3. CPSF6 binding defective CA mutants target significantly more into PBICs and less into PBSCs.
4. Integration into paused genes supports the connection between integration sites to HIV-1 latency as P-TEFb, Pol II pausing, and PAF-1 play a role in HIV-1 latency.

9. References

1. Ciazzi et al., Molecular Cells, 2021; 2. Koga et al., PLOS one, 2014; 3. Li et al., mBio, 2020; 4. Yu et al., Science, 2015; 5. Yang et al., PLOS Genetics, 2016; 6. Gao et al., Sci. adv., 2020

10. Acknowledgments:

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