3D Chromatin Capture of HIV Proviral Expression Reveals Epigenetic Regulators of HIV Latency

Megha S Srivatsa,[1], Jennifer Dan,[1,2] Nadejda Beliakova-Bethell,[1,3] Sarah A LaMere[1]

[1]University of California San Diego, La Jolla, CA 92093, USA, [2]La Jolla Institute for Immunology, La Jolla, CA 92037, USA, [3]VA San Diego Healthcare System and Veterans Medical Research Foundation, San Diego, CA 92161, USA
Author contact information: salamere@health.ucsd.edu; mesrivatsa@health.ucsd.edu

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Introduction

- During acute infection, **Human Immunodeficiency Virus (HIV)** integrates into the genome and becomes latent.
- **Proviral epigenetic modifications** likely play a role in **silencing during HIV latency**.[1,2] However, protein machinery underlying such epigenetic changes are poorly understood.
- The integrated **provirus interacts with host chromatin** at distant sites, known as **topologically associated domains (TADs)**, that are known targets **sites for host chromatin modifiers** and transcription factors.
- Identifying potential epigenetic regulators that interact with the provirus via TAD characterization **could present targets for an HIV cure strategies**.

Hypotheses

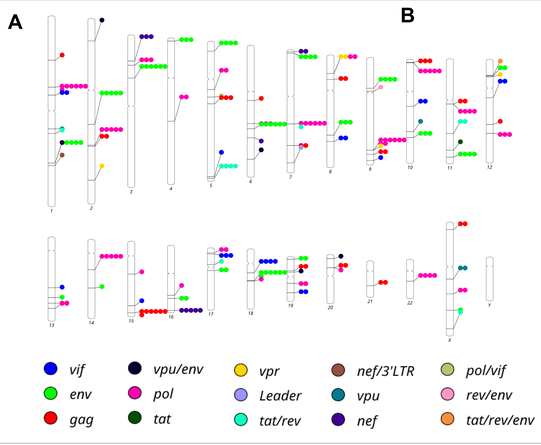
- 3D examination of host chromatin and proviral interaction sites in HIV infected CD4⁺ T cells will **reveal potential chromatin modifiers, functional pathways, and protein complexes** that facilitate HIV latency.
- Chromatin modifiers **co-localized** with proviral histone modifications **will reveal epigenetic mechanisms that impact proviral latency**.

Methods

1. **Clinical samples** were obtained through the Last Gift Rapid Autopsy Research Program.
2. **Single cell (sc) Hi-C chromatin capture** was performed on a primary CD4⁺ T cell latency model and **CD4⁺ T cells from lymph node of a person with HIV (PWH) with viral rebound** to identify TADs interacting with the provirus.
3. **Protein targets were identified** overlaying scHi-C TADs with known transcription factor binding sites using ReMap2022 ChIP-Seq of the human genome.
4. **Reactome** analysis of scHi-C transcription factors was used to identify **potential epigenetic pathways maintaining HIV latency**. Select prominent factors were searched in the **STRING** database to **identify functionally interactive proteins** overlapping with primary CD4⁺ T cell model Hi-C data.
5. **Selected transcription factors** alongside **histone modifications** were targeted by **CUT&Tag** in primary CD4⁺ T cell latency model and **spleen cells from a PWH on suppressive ART** to identify potential associations with epigenetic modifications.

Results

Overlap of **epigenetic regulators** and **histone modifications within the HIV provirus** both *in vitro* and **in primary tissue reveals novel regulatory elements of HIV latency** that may serve as therapeutic targets for an HIV cure.



Pathway name	#Entities found	#Entities total	Entities pValue	Entities FDR	#Reactions found	#Reactions total
RNA Polymerase II Transcription	335	1715	1.11E-16	8.99E-15	762	940
Epigenetic regulation of gene expression	88	343	1.11E-16	8.99E-15	105	119
Chromatin organization	83	269	1.11E-16	8.99E-15	70	105
Chromatin modifying enzymes	76	254	1.11E-16	8.99E-15	50	85
ESR-mediated signaling	60	257	1.11E-16	8.99E-15	100	114
SUMOylation	59	193	1.11E-16	8.99E-15	69	141
Estrogen-dependent gene expression	52	153	1.11E-16	8.99E-15	65	66
Regulation of endogenous retroelements	33	113	1.28E-12	6.52E-11	17	17
HDACs deacetylate histones	23	63	4.69E-11	1.97E-09	4	5
Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	23	64	6.35E-11	2.48E-09	45	51
PKMTs methylate histone lysines	19	48	6.03E-10	1.99E-08	12	22
SWI/SNF chromatin remodelers	15	31	2.76E-09	7.19E-08	20	20

Fig. 1. scHi-C identifies putative chromatin modifying factors contacting the HIV provirus. (**A**) Phenogram of **TADs between host chromatin and associated proviral regions** identified using scHi-C chromatin capture on a primary CD4⁺ T cell latency model. (**B**) **Transcription factors associated with TADs** were identified using Remap and subject to Reactome pathway analysis. Many transcription factors are **associated with epigenetic regulation.**

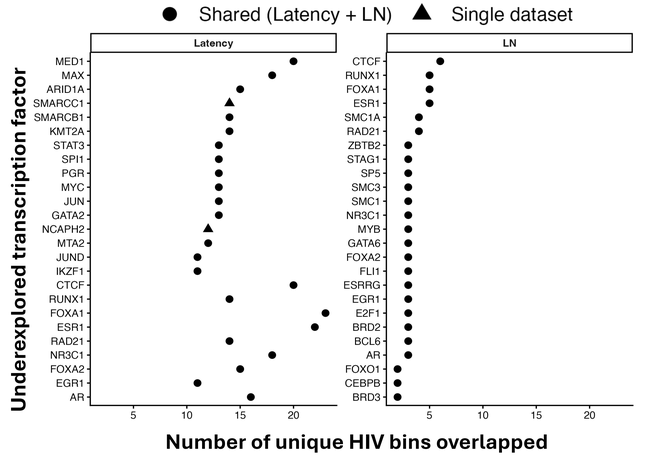


Fig. 2 Proviral interactors in a primary CD4+ latency model and CD4+ T cells isolated from a PWH show extensive overlap for understudied regulators. scHi-C of a primary CD4⁺ T cell latency model and **CD4⁺ T cells isolated from lymph node of a PWH after viral rebound** from ART discontinuation **reveals understudied putative transcription factors and chromatin remodelers regulating the provirus**.

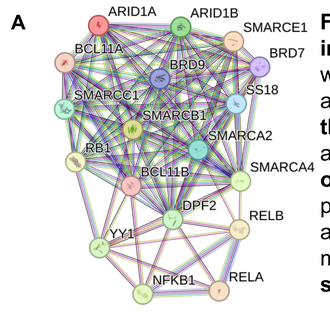
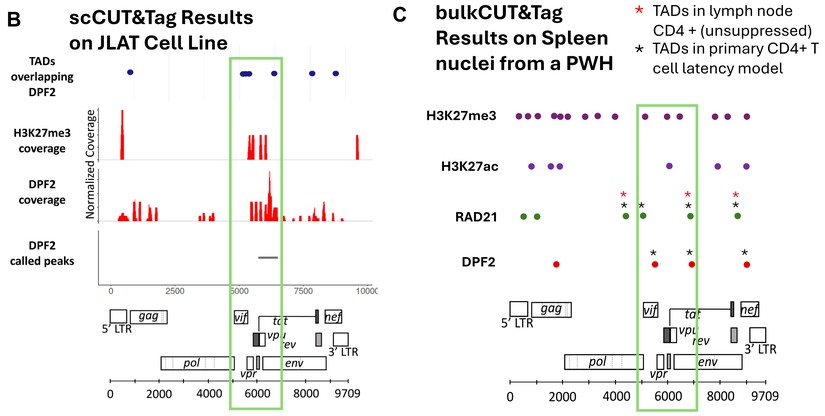


Fig. 3 Confirmation of DPF2 and RAD21 proviral interaction. Chromatin modifying protein **DPF2**[3,4,5] was subject to (**A**) STRING protein interaction analysis to identify **functionally associated proteins that overlap with the Hi-C data**, and (**B**) scCUT&Tag analysis, which returned **proviral binding sites that overlap with H3K27me3** in JLAT cells. (**C**) HIV proviral reads from CUT&Tag of putative targets **DPF2** and **RAD21** with **H3K27me3** and **H3K27ac** histone modifications **in spleen from a PWH on ART suppression share regional overlap with Fig. 3B**.



Conclusions and Future Directions

- The HIV provirus interacts with host chromatin at many sites across the host genome, which bind a variety of transcription factors involved in **functional pathways that impact proviral latency**, including **epigenetic regulation of endogenous retroviral elements**.
- Interaction analysis of BAF complex protein **DPF2** returned several transcription factors overlapping with scHi-C data **associated with the BAF chromatin modifying complex**,[6] which modulates HIV expression.[7]
- scHi-C analysis on a primary CD4⁺ T cell latency model and **in CD4⁺ T cells from lymph node of a PWH revealed many putative transcription factors interacting with the provirus**.
- **CUT&Tag** analysis in JLAT cells and **spleen cells from a PWH on suppressive ART** corroborate scHi-C findings of **DPF2 and RAD21** interacting with the provirus. Overlay of protein binding sites with H3K27me3 and H3K27ac suggests that DPF2 and RAD21 may maintain a 'poised' enhancer element and **a regulatory region of interactive chromatin architecture just before the envelope region on the provirus.**
- **Future investigation** may seek to corroborate longitudinal analysis of co-localized transcription factors with epigenetic marks from participants before and after initiating suppressive ART with *in vitro* activation of latent HIV. This would **enable causal inference of proviral latency**, and the dissection of molecular pathways that enable HIV persistence.

Acknowledgements and References

This work was supported by grants from the California HIV/AIDS Research Program H24BD7869 (LaMere), the Last Gift Cohort NIAIDAI169609 (P01 Smith), UCSD Altman Clinical and Translational Research Institute (ACTRI - UL1TR001442), and The James B. Pendleton Charitable Trust. Hi-C data was generated at the UC San Diego Center for Epigenomics. Sequencing data was generated at the UC San Diego IGM Genomics Center utilizing an Illumina X Plus that was purchased with funding from a National Institutes of Health SIG grant (#S10 OD026929).

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