Epigenomic characterization of a primary cell model of HIV latency

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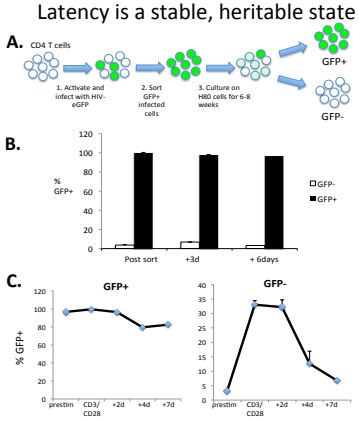
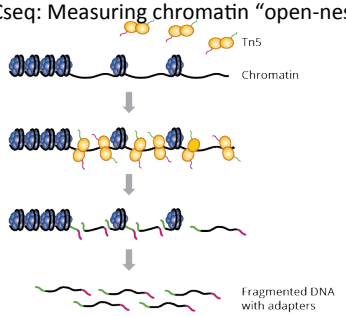
Abstract:
Transcriptional silencing of HIV in CD4 T cells generates a reservoir of latently infected cells that can reseed infection after interruption of therapy. As such, these cells represent the principal barrier to curing HIV infection, but little is known about their characteristics or regulation. To further our understanding of the molecular mechanisms of latency, we employed a primary cell model of HIV latency in which infected cells adopt heterogeneous transcriptional fates, with a subset of infected cells establishing viral latency. We characterized this model using Assay of Transposon-Accessible Chromatin sequencing (ATACseq). We observed that loss of viral gene expression is a stable and heritable phenotype that is maintained through multiple rounds of stimulation and expansion, suggesting a role for epigenetic maintenance of latency. Using ATACseq we found that cells in which latency is established exhibit a significantly more closed chromatin conformation, both within the HIV genome and across the host cell genome, indicating that latency is correlated with a global process of epigenomic modification and heterochromatin expansion. We also observed that latency reversing agents (LRAs) induced distinct patterns of chromatin opening in both the HIV and host cell genomes. Furthermore, we observed that latently infected cells exhibited elevated levels of specific repressive histone modifications, including H3K27me3. Altogether, these data demonstrate that latency establishment in primary CD4 T cells occurs preferentially in a subset of cells that exhibit expanded H3K27me3-associated heterochromatin, and that viral silencing is connected to global cellular epigenomic reprogramming. A deeper understanding of this process will likely lead to new therapeutic strategies for blocking the initiation or maintenance of latency.

Latency is a stable, heritable state



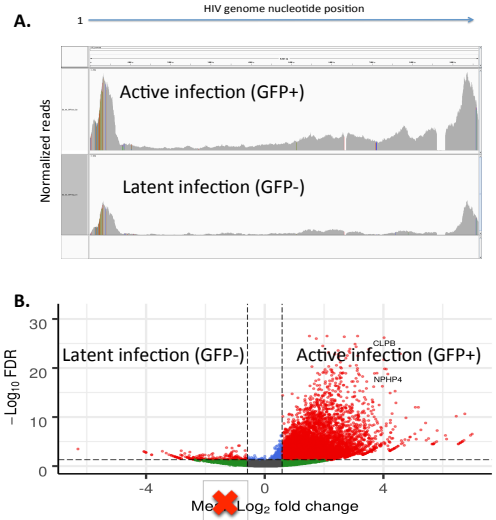
A. Overview of primary CD4 T cell latency model. B. Viral gene expression in sorted GFP+ or GFP- cells over time post sorting. C. Sorted GFP+/- cells were reactivated with anti-CD3/CD28 beads, and viral gene expression monitored over time post reactivation by flow cytometry

ATACseq: Measuring chromatin "open-ness"



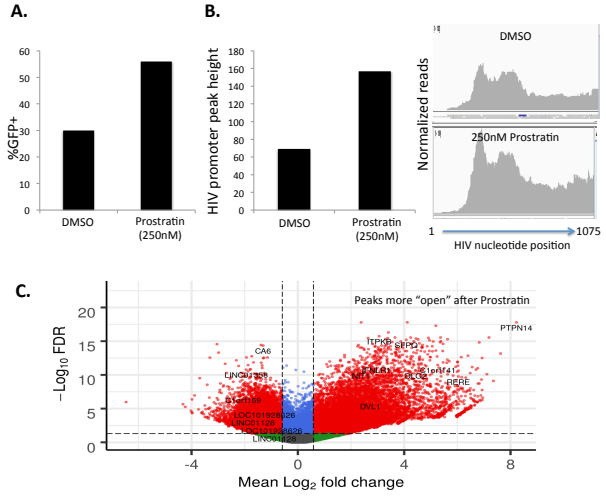
Assay of transposon-accessible chromatin sequencing (ATACseq) involves incubation of chromatin with a hyperactive Tn5 transposon. Insertion of Tn5 into open regions of chromatin causes DNA fragmentation and insertion of Illumina linker sequences. These fragments are then PCR amplified and sequenced to generate a topological map of open chromatin "peaks"

Latency is associated with a more closed chromatin structure



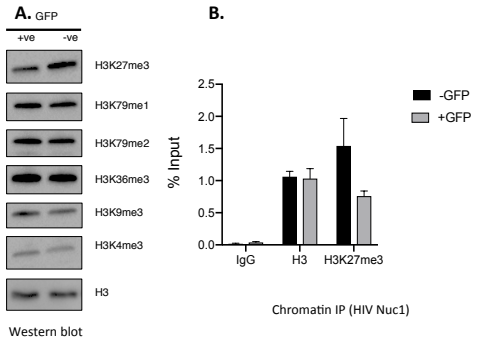
Latently infected (GFP+) and productively infected (GFP+) cells were sorted and analyzed by ATACseq. A. Reads were aligned to the HIV genome to determine accessibility of the proviruses in each cell population. The data show a more closed proviral conformation in latently infected cells B. Differentially accessible chromatin peaks were identified and quantified between latently infected (GFP-) and productively infected (GFP+) cells. We observed a more closed global chromatin conformation in the latently infected cells.

Epigenomic remodeling during latency reversal

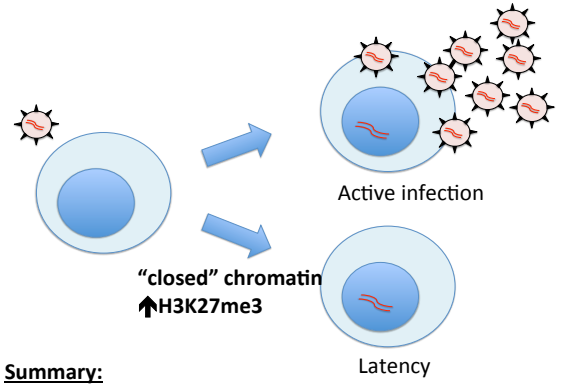


Latently infected primary CD4 T cells were stimulated with the PKC agonist Prostratin or control vehicle (DMSO). A. Flow cytometry of the infected cells demonstrates latency reversal in response to Prostratin. B. ATACseq reads were aligned to the HIV genome and the open-ness of the viral promoter was quantified by determining the proportion of mapped reads in each sample. C. ATACseq data was aligned to the the human genome and differentially open chromatin peaks after Prostratin stimulation were identified.

Latently infected cells have elevated H3K27me3



Latently infected (GFP-) and actively infected (GFP+) CD4 T cells were sorted and (A) western blotted for a panel of histone modifications. We observed elevated total H3K27me3 in the GFP- cells. (B) Chromatin from GFP+ and GFP- cells was abalyzed by ChIP for H3K27me3 modification at the HIV promoter (Nuc1).



Summary:

- Latency is a stable, heritable state
- Latently infected cells have more closed global chromatin conformation
- Latency reversal is associated with changes in chromatin accessibility at the HIV promoter and in thehost cell DNA
- Latently infected cells have elevated H3K27me3

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