Mechanisms of HIV Latency in CD4 T cells

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Facts about HIV Latency

- All patients contain latently infected cells
- Latently infected cells are rare (1/100,000 to 1/1,000,000)
- Latently infected cells are long-lived ($t_{1/2} = 44$ months)
CD4 T-Cell Differentiation, HIV Infection, and Latency Establishment

- **Non-Effector T (Th₀)**
  - CD45RA
  - CCR7
  - CXCR4

- **Effector T (Th₁,2,17, T_reg)**
  - CD45RA
  - CD45RO
  - CXCR4
  - ↑↑ CCR5

- **T_CM**
  - CD45RO
  - CCR7
  - CXCR4
  - ↑ CCR5

- **T_TM**
  - CD45RO
  - CXCR4
  - ↑↑ CCR5

- **T_EM**
  - CD45RA
  - CD45RO
  - CXCR4
  - ↑↑ CCR5

- **T_TD**
  - CD45RA
  - CD45RO
  - CXCR4
  - ↑↑ CCR5
The HIV Life Cycle

Immature Viral Particle → Budding

Mature Viral Particle

Receptor Binding
CD4 Receptor

Coreceptor

Rev
Tat
Nef

Integration

Reverse Transcription

Active Provirus

Latent Provirus

Nucleus
HIV Transcription Is Characterized by an Early, Tat-independent and a Late, Tat-dependent Phase

Early Tat⁻

“Short Transcripts”

U3 R U5

Exon 1

Tat

U3 R U5

Late Tat⁺

AAAAAA...
Establishment of HIV Latency: Early vs Late

- Early
  - Tat-independent

- Late
  - Tat-dependent

HIV Genome mRNA

Time Post Infection

Threshold
Establishment of HIV Latency: Early vs Late

HIV Genome mRNA

Time Post Infection

Threshold

Early Tat-independent
Late Tat-dependent
Establishment of HIV Latency: Early vs Late

- Early: Tat-independent
- Late: Tat-dependent

HIV Genome mRNA vs Time Post Infection

Threshold

Stimulation
Establishment of HIV Latency: Early vs Late

- Early
  - Tat-independent
- Late
  - Tat-dependent

HIV Genome mRNA

Threshold

Time Post Infection
Mutations in the Tat-TAR Axis Lead to HIV-1 Latency

A point mutation in the HIV-1 Tat responsive element is associated with postintegration latency

Stephane Emiliani*, Carine Van Lint*, Wolfgang Fischle*, Peter Paras, Jr., Melanie Ott*, John Brady‡, and Eric Verdin*§

PNAS 1996; 83:6377

Mutations in the tat Gene Are Responsible for Human Immunodeficiency Virus Type 1 Postintegration Latency in the U1 Cell Line

Stephane Emiliani, Wolfgang Fischle, Melanie Ott, Carine Van Lint, Carol Ann Amella, and Eric Verdin

J. Virol. 1998; 72:1666
Regulation of HIV Basal Transcription by Cis- and Trans-Acting Factors

**Integration Site (Cis-)**
- Chromatin and Associated Factors (HDACs, Histone Methyltransferases...)

**T-Cell Activation (Trans-)**
- Transcription Factors (NF-κB, NFAT...)

![Diagram showing the regulation of HIV basal transcription by cis- and trans-acting factors.](image-url)
Regulation of HIV Basal Transcription by Cis- and Trans-Acting Factors

Integration Site (Cis-)

Chromatin and Associated Factors (HDACs, Histone Methyltransferases....)

T-Cell Activation (Trans-)

Transcription Factors (NF-κB, NFAT,....)

U3 R U5

+ -

+ -

+ -
Heterochromatin and T Cell Activation

From *Ultrastructure and Function of Heterochromatin and Euchromatin*
J. H. Frenster
Regulation of HIV Basal Transcription by Cis- and Trans-Acting Factors

Integration Site (Cis-)

Chromatin and Associated Factors (HDACs, Histone Methyltransferases...)

DNA methylation

T-Cell Activation (Trans-)

Transcription Factors (NF-κB, NFAT....)

miRNA
Heterogeneity of HIV Promoter Activity After Infection of Peripheral Blood Mononuclear Cells

- Uninfected
- Infected
- R2-Gated

Jurkat
- VSV.G
- HIV-Env

PBMC
- R2

FL2H

Counts

GFP
Heterogeneity in HIV Expression: Cis- vs. Trans- Effects

- LTR-GFP
  - MFI
  - Histogram
- LTR-Luciferase
  - LRU
  - Histogram
- Scatter plot: LTR-GFP (MFI) vs. LTR-Luciferase (LRU)
  - $r^2 = 0.052$
  - $p = 0.19$
Selection of Cells Latently Infected with HIV

HIV-R7/E−/GFP

GFP-positive Cells (%) vs. Clone with 0.5-2% Basal
Selection of Cells Latently Infected with HIV

HIV-R7/E−/GFP

100

GFP-positive Cells (%)

Clone

Basal

TPA
Latent HIV integration sites in J-Lat cells

1. Heterochromatin mediated silencing ~10%
2. Transcriptional interference ~10%
3. Virus integration-mediated mutagenesis <5%
4. Gene deserts, long intergenic regions ~15%
5. Unknown (integration within genes) ~60%
Methylation-mediated Repression
via MBD2/Mi-2/NurD
DNA Methylation of HIV Promoter in Latently vs Productively Infected Cells

GFP Fluorescence

Side Scatter

GFP-Negative

GFP-Positive

CpG Island 1

CpG Island 2

CpG Island 1

CpG Island 2
HIV CpG Islands Are Methylated in Latently Infected Primary CD4+ Cells

21 days post infection
Conclusions

-HIV Latency is established at the transcriptional level in primary memory T cells

-Cis-acting and Trans-acting mechanism are likely to contribute to the establishment of HIV latency in primary T cells

-While trans-acting factors are likely to be homogeneous within a homogeneous lymphocyte population (i.e. resting T_{CM}), different integration sites are likely to suppress HIV transcription via distinct mechanisms. This is due to the variety of repressive chromatin environments that exists in cells.

-HIV latency is therefore likely to be heterogeneous (therapeutic implications)

-The HIV genome is methylated in a large fraction of latently-infected cells (both transformed and primary lymphoid cell models)

-Inhibitors of methylation potently synergize with trans-acting factors (TNF, prostratin) to reactivate latent HIV

-Future efforts will need to better define the mechanism of HIV latency in primary lymphoid cells either isolated from patients or from appropriate in vitro model systems.