Defective HIV-1 Proviruses from Resting CD4+ T Cells Can Be Transcribed and Translated

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The HIV-1 latent reservoir is the major barrier to cure

Greene and Siliciano, Cold Spring Harb Perspect Med, 2011

The HIV-1 Latent Reservoir is a stable reservoir of reversibly quiescent integrated HIV-1 proviruses.

Siliciano et al., Nat Med, 2003

The major barrier to the cure of HIV-1 is the persistence of latently infected resting CD4\(^+\) T Cells despite long-term combination ART.
Measurement of the size of the latent reservoir is complicated

The quantitative viral outgrowth assay (qVOA) measures the number of latently infected cells with replication competent proviruses.

Eriksson et al., PLoS Pathog, 2013

The qVOA underestimates the true size of the latent reservoir.

Bruner et al., Trends in Microbiology, 2015
The majority of the HIV-1 proviruses are defective

Large Internal Deletions

The primary types of defective proviruses in resting CD4+ T Cells are large internal deletions and APOBEC mediated G-to-A hypermutation.

Ho et al., Cell, 2013
Can defective proviruses be transcribed?

Transcription of defective proviruses may complicate the measurement of the latent reservoir. Are we measuring replication competent virus becoming reactivated?

Bullen et al., Nature Med 2014

Margolis et al., Nature 2012

Margolis et al., JID 2014
Patient derived defective HIV-1 proviruses were synthesized by de novo gene synthesis following full length sequencing.

Ho et al., Cell, 2013

Intracellular Assays:
- Cell associated HIV-1 RNA
- Intracellular Gag Staining FACS

Supernatant Assays:
- Supernatant HIV-1 RNA
- Supernatant p24 ELISA
Selection of Candidate Defective Proviruses

![Diagram showing the selection of candidate defective proviruses with open reading frames for Gag, Tat, and Rev proteins. The diagram includes a table indicates the presence or absence of open reading frames for each candidate.]

**Candidates**
- **48C8**
- **4F12**
- **E44E11**
- **E39P14**
- **4F11**
- **45E6**
- **42B6**
- **39G2**
- **2G10**
- **31G4**
- **19B3**
- **TatM**
- **RevM**
- **TatM+RevM**
- **17TB4_4F11**

**Open Reading Frames**
- **Intact**
- **Defect**

**Protein Expression**
- **Gag**
- **Tat**
- **Rev**
Defective HIV-1 proviruses can be transcribed.

Transcription of Total HIV-1 mRNA

qRT-PCR of Cell Associated HIV-1 RNA. All HIV-1 transcripts are normalized to GFP transfection efficiency control.
Defective HIV-1 proviruses can make protein.

Healthy Donor CD4+ T Cells were transfected with defective HIV-1 proviruses and stained for HIV-1 gag protein.

Expression was measured by FACS.
Defective HIV-1 proviruses may be translated and released

P24 Growth Kinetics

**Hypermutated**

**Large Internal Deletion**

**MSD/Packing Loop Deletion**

**Point Mutation**
Conclusions

- Defective proviruses can be transcribed.
  - Transcription is largely dependent on an intact \textit{tat} gene.
- Some defective proviruses can produce protein.
  - Viral protein production is largely dependent on an intact \textit{tat} and \textit{rev} gene.
- Defective proviruses should be considered when measuring cell-associated HIV-1 RNA.
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