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## Patient-derived defective HIV-1 proviruses containing large internal deletions can be transcribed and translated

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**Background:** Despite antiretroviral therapy (ART), human immunodeficiency virus-1 (HIV-1) persists as integrated proviruses primarily in resting CD4+ T cells as the major barrier to cure. The majority of these proviruses are defective, containing large internal deletions or APOBEC-mediated G-to-A hypermutations. With intact promoter function and lack of epigenetic silencing, some defective proviruses can potentially be transcribed. Transcription of defective proviruses may complicate the measurement of latency reversal using PCR-based assays, such as cell-associated RNA quantification. We have previously reported that hypermutated proviruses can be transcribed from patient resting CD4+ T cells. However, it remains unclear whether proviruses containing large internal deletions can be transcribed. Identification of the proviral components (single genes such as tat and rev or specific region of the proviral genome) required for defective proviruses to be transcribed may help to design a more accurate assay for the measurement of latency reversal.

**Methods:** To identify the proviral genes essential for viral transcription, we constructed proviral clones defective in gag, tat and/or rev by site-directed mutagenesis of the NL4-3 reference strain. To identify the type of defective provirus which can be transcribed, we reconstructed full-length defective proviral clones containing large internal deletions (encompassing the packaging signal, 5' or 3' of the proviral genome) and hypermutations. These clones were isolated from resting CD4+ T cells of HIV-1-infected individuals under suppressive ART through limiting-dilution PCR and reconstructed through de novo full-length genome synthesis. Primary CD4+ T cells were transfected with proviruses and activated by CD3/CD28 co-stimulation. After DNase treatment, cell- associated HIV-1 RNA was measured by quantitative RT-PCR. HIV-1 protein expression was measured by flow cytometry of the Gag protein.

**Results:** We reconstructed 11 full-length defective proviral clones from HIV-1-infected individuals. Defective proviruses with intact tat genes can produce HIV-1 RNA at lower but significant levels following CD3/CD28 co-stimulation. These defective proviruses are capable of producing HIV-1 viral proteins at measurable but lower levels than the NL4-3 reference strain.

**Conclusions:** Defective HIV-1 proviruses can be transcribed and translated following stimulation. HIV-1 transcription from defective proviruses, especially those containing an intact tat, should be considered in the measurement of latency reversal.

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