Reversal of a Latency-Like Phenotype Using The Small Antigen of the Hepatitis *Delta* Virus, A Counterintuitive Way to Activate Latent HIV Infected Cells

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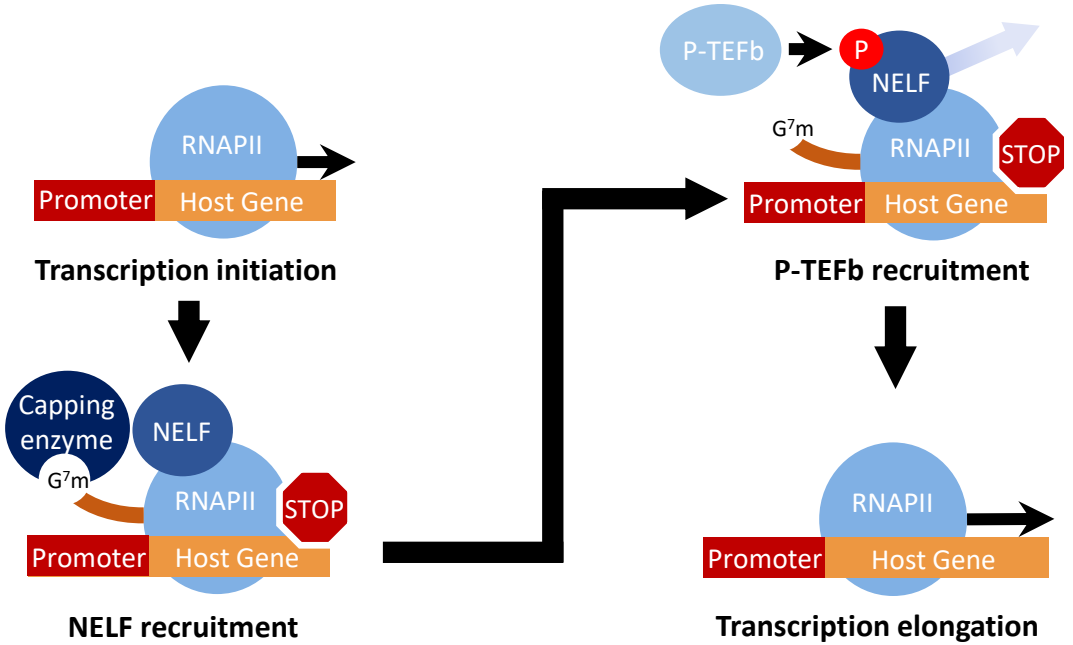
BACKGROUND

PROMOTER-PROXIMAL PAUSE



Promoter-proximal pause: A halt in transcription elongation induced by the interaction of **NELF** with RNAPII shortly after promoter clearance[1].

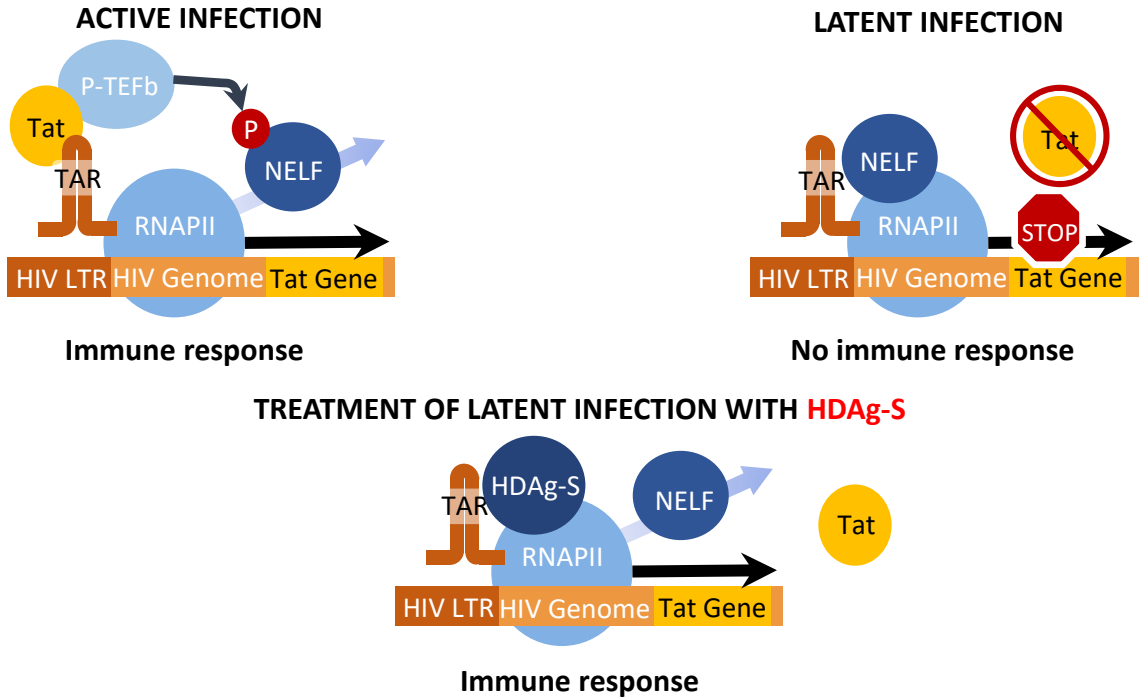
This pause allows **capping** of nascent transcripts[2], maintenance of a **permissive chromatin landscape** around active promoters[3] and a **quick transcriptional response** from stimulus-responsive genes[4].

This pause also affects transcription of proviral **HIV**[5].

The small **hepatitis *delta* antigen (HDAg-S)**, one of the two proteins produced by the **hepatitis *delta* virus (HDV)**, was shown to stimulate RNAPII processivity[6].

As HDAg-S and NELF share a **sequence similarity**, it has been suggested that HDAg-S stimulates RNAPII processivity by **competing with NELF** for a common surface on RNAPII[6].

HIV LATENCY



The promoter-proximal pause is one of the mechanisms by which HIV enters **latency**[5].

Levels of **Tat** in **latently infected cells** are not sufficient to efficiently recruit P-TEFb to the stalled RNAPII[7].

Latently infected cells are not targeted by antiretroviral therapies and create **viral reservoirs** that are reactivated upon treatment interruption[8].

In HIV-infected patients, reducing the promoter-proximal pause could reactivate latent HIV reservoirs so all HIV-infected cells could be targeted by antiretroviral therapies.

HYPOTHESIS

Using HDAg-S as a NELF competitor could lead to transcriptional reactivation of latently infected cells.

RESULTS

EFFECTS OF HDAg-S ON HEK 293T CELLS

METHODOLOGY

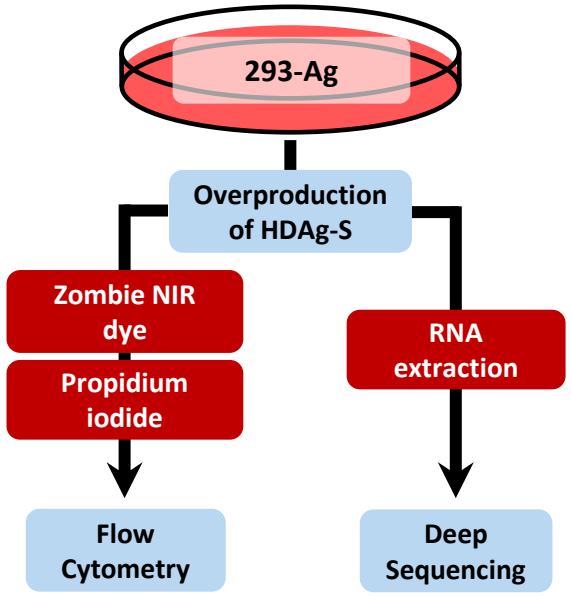


FIGURE 1

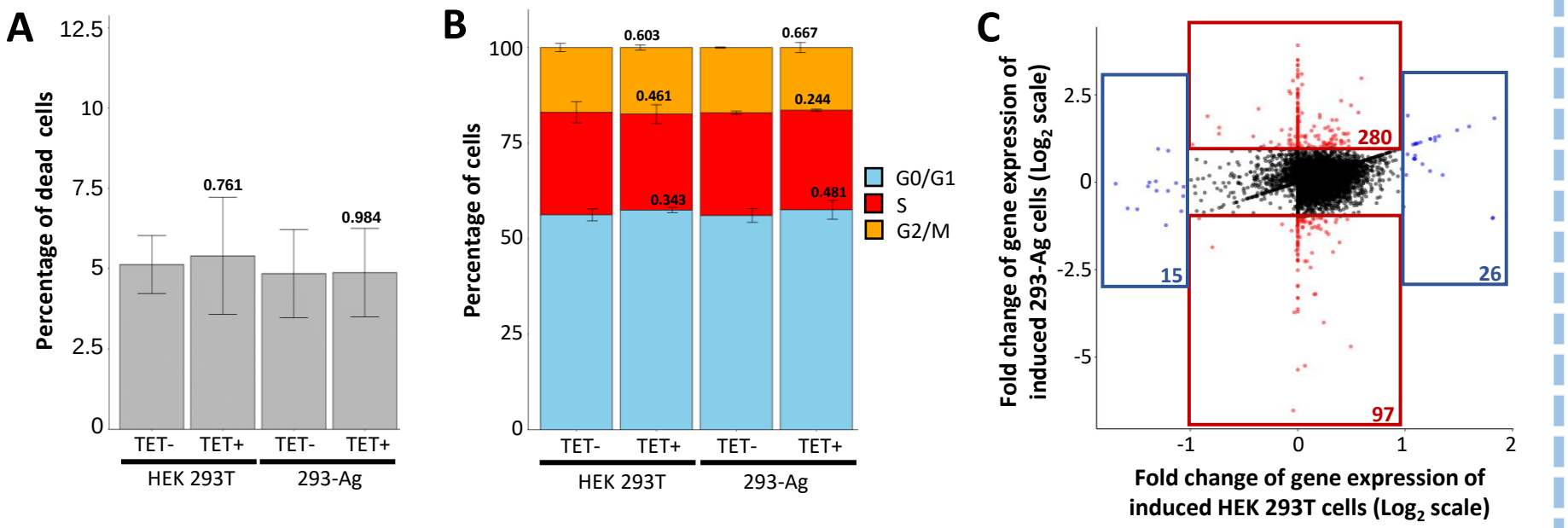


Figure 1. HDAg-S expression has minimal effect on HEK 293T cells.
293-Ag cells overexpress HDAg-S when induced with tetracycline (+TET). HEK 293T cells were used as a negative control for HDAg-S expression. (A) Amount of cells stained with Zombie NIR in presence or absence of HDAg-S. (HEK 293T: n=6; 293-Ag: n=3) (B) Cell cycle analysis of cells expressing HDAg-S or not. (HEK 293T n=4; 293-Ag n=2) (C) Gene expression of induced HEK 293T and 293-Ag cells was compared to uninduced HEK 293T cells. Genes with at least a two-fold difference of expression between uninduced HEK 293T cells and induced HEK 293T cells are shown in blue. Genes with at least a two-fold difference of expression between uninduced 293-Ag and induced HEK 293T cells are shown in red. (n=1) (A-B) Induced cells were compared with their uninduced counterpart in a t test, the resulting p-value is indicated above the respective error bar.

RESULTS

EFFECTS OF HDAg-S ON HIV TRANSCRIPTION

METHODOLOGY

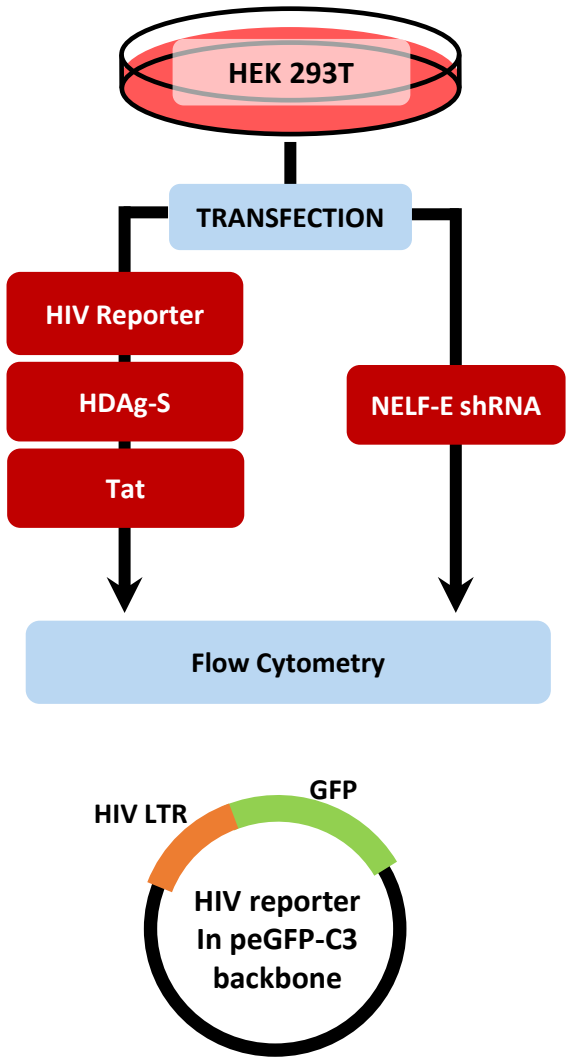


FIGURE 2

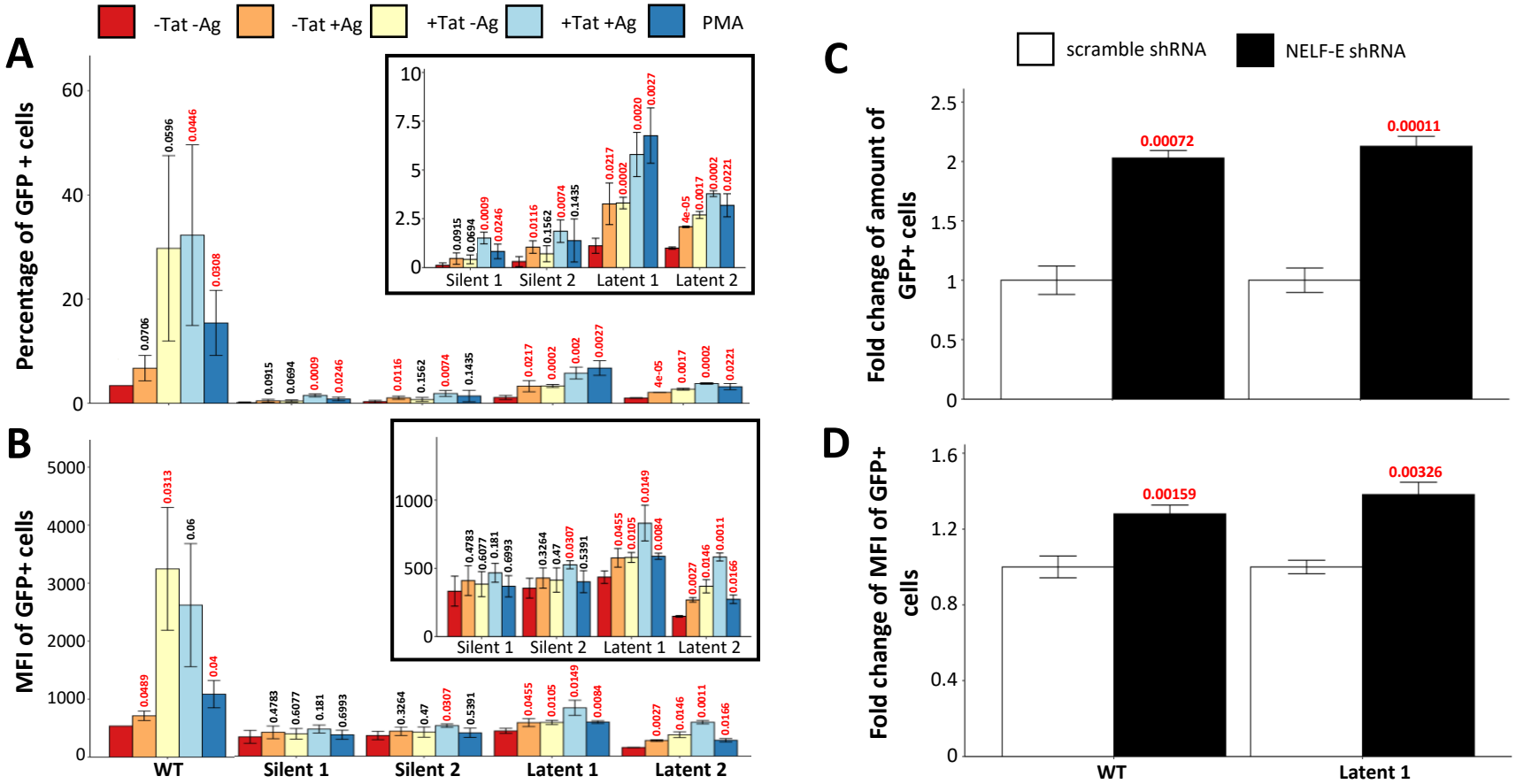


Figure 2. HDAg-S and Tat have similar effects on transcription of latent HIV mutants.

(A) Amount of GFP positive cells in different transfection conditions (n=4). (B) Mean fluorescence intensity (MFI) of cells in different transfection conditions (n=4). (A-B) Insets display zoomed in data for silent and latent mutants. Conditions compared to respective -Tat -Ag condition, resulting p-values are written above respective sample. (C) Amount of GFP positive cells from samples co-transfected with HIV reporters and shRNA against NELF-E (n=3). (D) MFI of GFP positive cells from samples co-transfected with HIV reporters and shRNA against NELF-E (n=3). For all assays: red value: p-value < 0.05.

CONCLUSIONS

- HDAg-S is not significantly toxic to HEK 293T cells.
- HDAg-S stimulates transcription on latent HIV reporters, possibly by hindering the pause.
- **HDAg-S and Tat work in synergy** to further stimulate transcription of latent HIV.
- **HDAg-S might act as a kick-starter** to produce Tat, which will then stimulate HIV transcription and induce HIV replication.

VIDEO ABSTRACT

https://youtu.be/IPnnDGbpGxM

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NSERC CRSNG

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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