Identification and characterization of the HIV-1 antisense transcript Ast functional domains involved in proviral epigenetic silencing

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BACKGROUND

HIV-1 latency occurs via epigenetic modifications that lead to nucleosome assembly and transcriptional silencing of the proviral 5'LTR. HIV-1 expresses an antisense transcript (*Ast*) from a negative sense promoter within the 3'LTR. We reported that *Ast* recruits the Polycomb Repressor Complex 2 (PRC2) to the HIV-1 5'LTR, promoting trimethylation of lysine 27 on histone H3 (H3K27me3), nucleosome assembly and transcriptional silencing[1]. Here, we sought to further dissect these events and to identify domains and motifs of *Ast* that play a role in promoting HIV-1 latency.

METHODS

We generated a panel of *Ast* mutants, which we stably transduced into the Jurkat cell-based model of latency (Jurkat E4), which contain a GFP reporter for ease of measurement. We used a combination of functional and molecular assays to identify the domains and motifs of *Ast* that are involved in promoting HIV-1 latency. We also performed pull down and mass spectrometry studies to identify additional transcriptional and epigenetic silencers associated with *Ast*.

RESULTS

Identification of the *Ast* domain and motifs involved in the interaction between *Ast* and the proviral 5'LTR

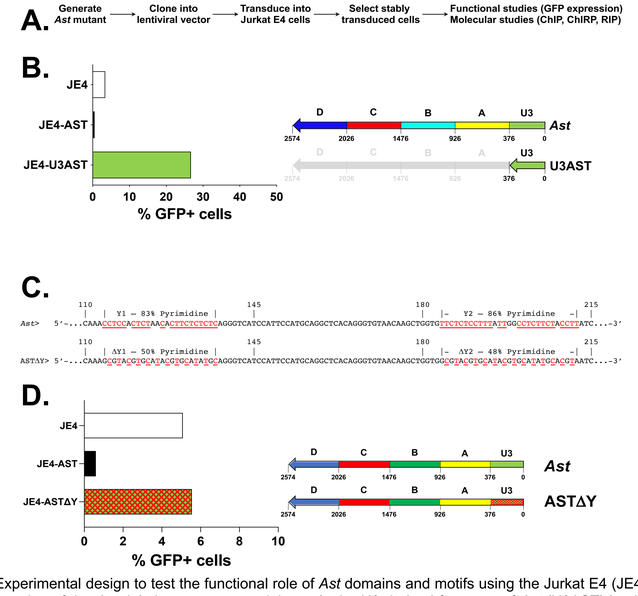


Fig. 1. A: Experimental design to test the functional role of *Ast* domains and motifs using the Jurkat E4 (JE4) model. B: Stable expression of the *Ast* deletion mutant containing only the U3-derived fragment of *Ast* (U3AST) leads to latency reversal in JE4 cells. C: Nucleotide substitutions within the pyrimidine-rich motifs (Y1 and Y2) in the U3-derived region of *Ast* reduced pyrimidine content to 50% and 48%, respectively (ΔY1 and ΔY2). D: Stable expression of the *Ast* substitution mutant containing ΔY1 and ΔY2 motifs (ASTΔY) leads to latency reversal in JE4 cells.

The *Ast* polypyrimidine motifs mediate interaction with the 5'LTR

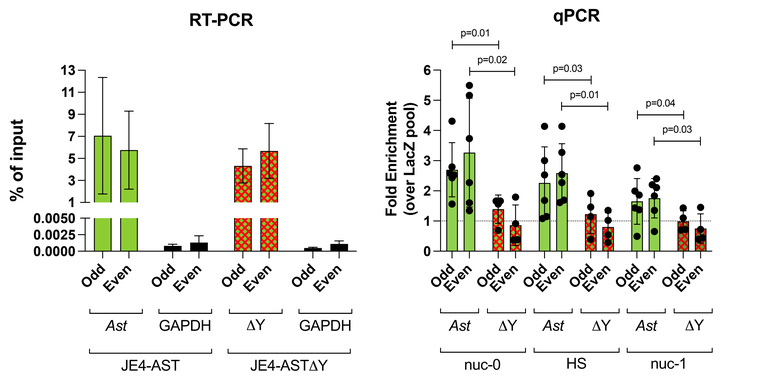


Fig. 2. *Ast* is recruited to the 5'LTR via pyrimidine-rich stretches. Chromatin isolation by RNA precipitation (ChIRP) assays were performed using biotinylated oligos complementary to *Ast* and *LacZ* RNA. Quantitative PCR assessing the specificity and the enrichment of *Ast* or GAPDH RNA (A), and three regions of the proviral 5'LTR (nuc-0, hypersensitive site (HS), and nuc-1) from JE4 cells expressing wildtype *Ast* (JE4-AST) or ASTΔY (JE4-ASTΔY) (B).

Identification of the *Ast* domain and motifs interacting with PRC2

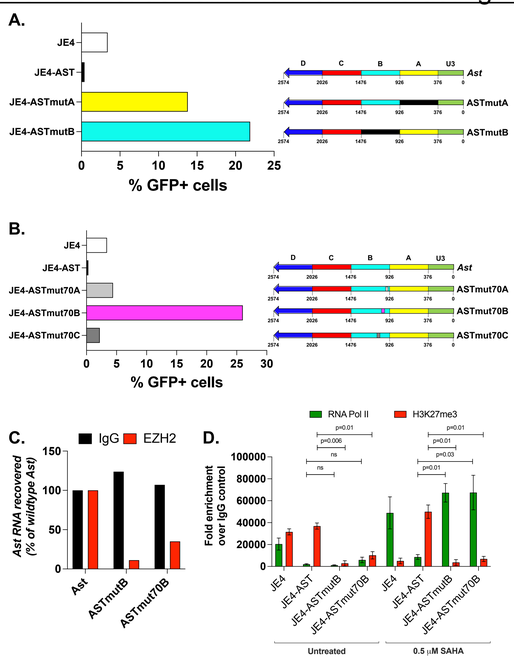


Fig. 3. A: Substitution of segment B (ASTmutB) and, to a lesser extent, segment A (ASTmutA) of *Ast* with an unrelated sequence profoundly impacts its silencing function. B: Substitution of 70-nt surrounding a G-quadruplex motif within segment B of *Ast* (ASTmut70B) profoundly impacts its silencing activity. C: Both ASTmutB and ASTmut70B show markedly reduced interaction with the EZH2 subunit of PRC2 in RIP assays. D: JE4 cells stably transduced with ASTmutB or ASTmut70B display markedly reduced levels of H3K27me3 in the Nuc-1 region of the HIV-1 5'LTR compared to JE4 cells stably transduced with WT *Ast* (JE4-AST). E: Both ASTmutB and ASTmut70B show markedly increased GFP expression both at baseline and after SAHA treatment compared to parental JE4 and JE4-AST cells.

Cooperative role of segments C and D in the activity of *Ast*

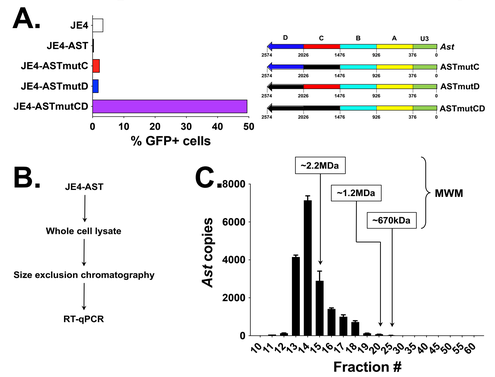


Fig. 4. A: Substitution of either segment C or D (ASTmutC and ASTmutD) does not significantly alter the function of *Ast*. However, concurrent substitution of both segments of *Ast* (ASTmutCD) profoundly affects its silencing function, indicating cooperativity. B: Experimental design to identify size of molecular complexes associated with *Ast* RNA. C: Cell lysates from Jurkat cells expressing *Ast* were fractionated over a size exclusion column, and fractions were analyzed by RT-qPCR. *Ast* RNA co-purifies predominantly in fractions containing molecular complexes of >2.2MDa in size, suggesting interaction with other RNA binding complexes in addition to PRC2.

Identification of additional *Ast*-binding partners

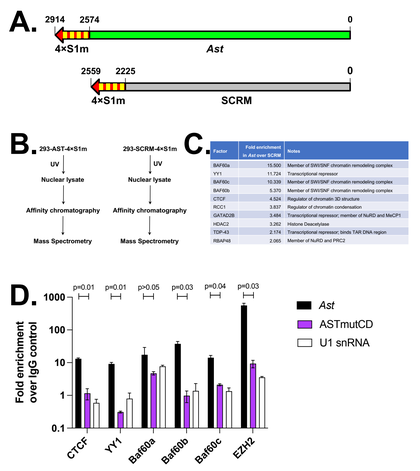


Fig. 5. Identification of new *Ast* binding partners. A: Schematic representation of the aptamer (4×S1m)-tagged *Ast* and control (SCRM) RNA utilized in affinity purification. B: 4×S1m-tagged *Ast* or SCRM RNA were transfected into 293T cells. *Ast* and SCRM RNA were purified from cell lysates with streptavidin beads, and co-purifying proteins were identified by mass spectrometry. C: Enrichment of transcriptional and epigenetic silencers enriched in *Ast* RNA pulldowns compared to SCRM pulldowns. D: RIP assays validating the interaction between *Ast* and some of the targets identified in panel C. These interactions are markedly reduced in cells expressing ASTmutCD.

CONCLUSIONS

These studies provide molecular and functional evidence that the HIV-1 antisense transcript *Ast* promotes viral latency by bridging PRC2 and additional host factors to the 5'LTR, leading to its transcriptional suppression. These results further support the use of *Ast* RNA in the context of 'block and lock' HIV-1 cure strategies.

REFERENCES

1. Zapata et al. *Virology,* **506:**34-44, 2017.

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