The HIV-1 Antisense Transcript *Ast* Induces Viral Latency through Multiple Silencing Pathways

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BACKGROUND

Antiretroviral therapy can suppress HIV replication but is unable to eradicate the virus. One reason is that HIV persists in a small number of resting memory T cells where HIV has been transcriptionally silenced. We previously reported that the HIV-1 antisense transcript (Ast) induces the establishment and maintenance of HIV-1 latency via recruitment of the Polycomb Repressor Complex 2 (PRC2) to the HIV-1 5'LTR, leading to trimethylation of lysine 27 on histone H3 (H3K27me3), nucleosome assembly and transcriptional silencing. However, the mechanisms whereby Ast promotes HIV latency remain incompletely understood. A systematic analysis of functional elements in Ast and it's potential binding protein complex will provide an important insight into the development of novel therapeutically approaches.

METHODS

- Ast mutants were generated by mutagenesis.
- Affinity purification- Mass spectrometry was used to identify Ast binding protein.
- RNAseq was used to look at the effect of Ast on host response.

RESULTS

Mapping of functional domains in the *Ast* RNA using latently infected Jukat E4 cells

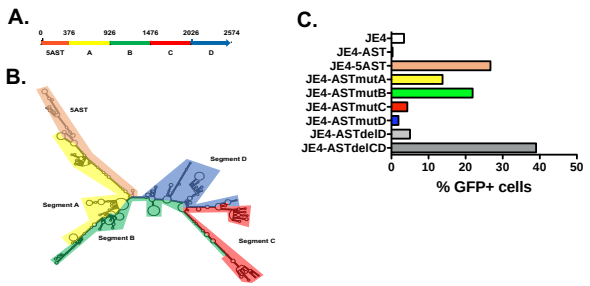


Fig. 1. Predicted secondary structure of *Ast* and location of mutants. A: linear map of *Ast* showing the five segments: 5AST, A, B, C and D. B: predicted secondary structure of *Ast* with the five segments highlighted in the same colors as in the linear map. C: The seven *Ast* mutants were stably transduced in JE4 cells to assess their impact on the HIV-1 silencing compared to the wildtype *Ast* sequence.

Ast binds to HIV 5'LTR promoter

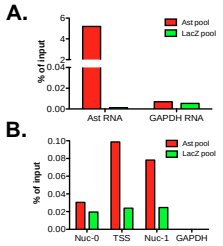


Fig. 2. Chromatin isolation by RNA precipitation (ChIRP) assay. RT-PCR assessing precipitation of *Ast* and *GAPDH* RNA with biotinylated oligos complementary to *Ast* and *LacZ* RNA (A.). Quantitative PCR assessing the amount of HIV-1 5'LTR (Nuc-0, transcription start site TSS, and Nuc-1 regions) and GAPDH promoter chromatin co-precipitating with *Ast* and *LacZ* RNA (B.).

Identification of PRC2-binding sequence in the *Ast* RNA segment B

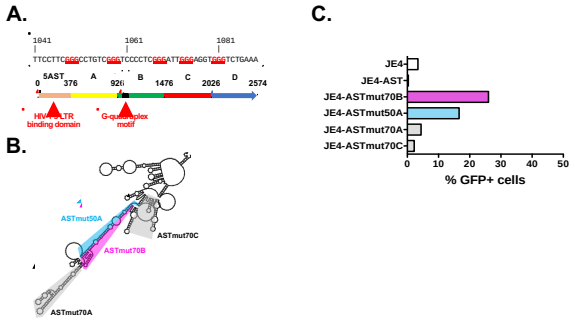


Fig. 3. A: position of the putative [G3N3-7]5 G-quadruplex motif in segment B of *Ast*. B: enlarged view of the lower left "stem-loop" of *Ast* (see Fig. 1). The sequences involved in the four substitution mutants are shown in gray (ASTmut70A and ASTmut70C), violet (ASTmut70B) and light blue (ASTmut50A) shaded areas. C: Effect of substitution mutants within Segments A and B of *Ast*.

G-quadruplex motif are responsible for interaction with PRC2

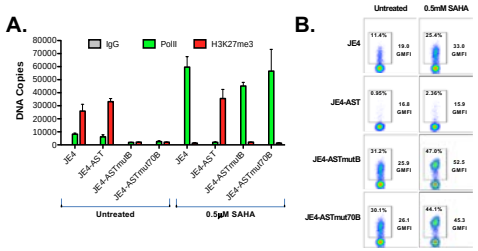
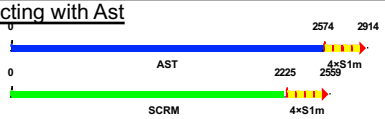


Fig. 4. A: ChIP analysis of JE4, JE4-AST, JE4-ASTmutB and JE4-ASTmut70B cells before and after SAHA treatment. Samples were immunoprecipitated with antibodies to RNA Pol II and H3K27me3, or with IgG isotype, and analyzed with primers specific for Nuc-1. B: flow cytometry analysis showing GFP expression in the four cell lines before and after SAHA treatment.

Identification of additional repressive epigenetic and transcription factors interacting with Ast



Category	Factor	Ast/SCRM	Notes
Epigenetic & Transcription Silencers	BAF60a	15.500	Member of SWI/SNF chromatin remodeling complex
	YY1	11.724	Transcriptional repressor
	BAF60c	10.339	Member of SWI/SNF chromatin remodeling complex
	BAF60b	5.370	Member of SWI/SNF chromatin remodeling complex
	CTCF	4.524	Regulator of chromatin 3D structure
	RCC1	3.837	Regulator of chromatin condensation
	GATAD2B	3.484	Transcriptional repressor; member of NuRD and MeCP1
	HDAC2	3.262	Histone Deacetylase
	TDP-43	2.174	Transcriptional repressor; binds TAR DNA region
	RBAP48	2.065	Member of NuRD and PRC2
	DKC1	5.946	Pseudouridine synthase 1
RNA Modification	NOP58	4.500	Member of C/D box complex; 2'-O-ribomethylation
	FTSJ3	3.300	RNA methyltransferase
	FBL	2.151	RNA methyltransferase; member of C/D box complex

Fig. 5. Identification of new *Ast* binding partners. Aptamer (4S1m)-tagged *Ast* and control (SCRM) RNA (top schemes) were expressed in 293T cells. The two RNA were purified with streptavidin beads, and proteins bound specifically to *Ast* compared to SCRM were identified by mass spectrometry (Table).

Validation of *Ast* binding proteins

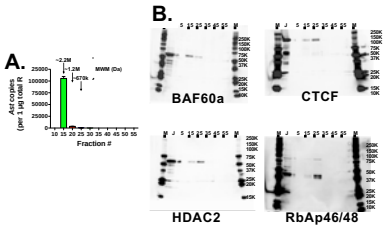


Fig. 6. Co-purification of *Ast* with epigenetic and transcription silencers. Cell lysates from Jurkat cells expressing *Ast* were fractionated over a size exclusion column. Fractions were first analyzed by RT-PCR for *Ast* RNA (A) and then by western blot (B) with antibodies directed against some of the proteins that were identified by mass spectrometry in the *Ast* pulldown experiment.

Effect of *Ast* on host gene expression

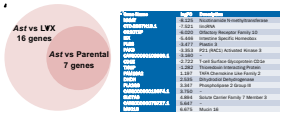


Fig. 7. RNAseq analysis of gene expression in cells stably transduced with *Ast* versus cells stably transduced with an empty lentivirus (LVX) or versus parental cells. The analysis was performed in three different cell backgrounds (CEM, H9 and Jurkat). Left: Venn diagram showing the number of host genes differentially expressed between *Ast* vs. LVX cells, and between *Ast* vs. parental cells in all three backgrounds. Right: list of differentially expressed genes in *Ast* cells vs. LVX and parental cells in all three backgrounds.

Conclusions

We have identified the 5'LTR- and PRC2-binding domains of *Ast*, as well as additional transcriptional silencers recruited by *Ast*. Further studies are on the way to characterize the role of *Ast* binding proteins in HIV latency.

References

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2. Wang et al. *Molecular Cell* **65:**1056-1067, 2017.
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Funding

NIH Grants AI120008, AI124776, AI134151, and amfAR Grant 109612-62-RGRL to FR. NIH Grants AI078859, AI074410, and AI043894 (to FK).