Reactivation of HIV Latently Infected T cells by Targeting Tat IRES Translation

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Georges Khoury[1], Sri Ramarathinam[2], Charlene Mackenzie[1], David Yurick[1], Con Sonza[1], Tony Purcell[2], Damian Purcell[1]

[1]Department of Microbiology and Immunology, University of Melbourne at Peter Doherty Institute for Infection & Immunology, VIC, 3010. [2]Department of Biochemistry & Molecular Biology, Monash University, VIC, 3800.

Introduction

In latently infected memory CD4+ T cells, the HIV provirus predominantly integrates into introns of transcriptionally active genes where it is subject to transcriptional interference. The resulting read-through transcription and splicing can incorporate HIV RNA into mature cellular RNAs. HIV-1 proviruses may show stochastic fluctuations in expression depending on levels of Tat protein. Activating alternative splicing either at the HIV A3-A7 3' splice sites or at the cellular gene flanking the HIV provirus would lead to the incorporation of Tat-exon 2, which codes for a 72 amino acids Tat protein. Studies from our team have revealed a novel expression pathway of Tat from chimeric cellular-HIV RNAs, mediated by an Internal Ribosome Entry Site (IRES) present in Tat-exon2. To understand the function of Tat IRES, a detailed knowledge is required of the RNA structure and the cellular factors that binds to these chimeric RNAs.

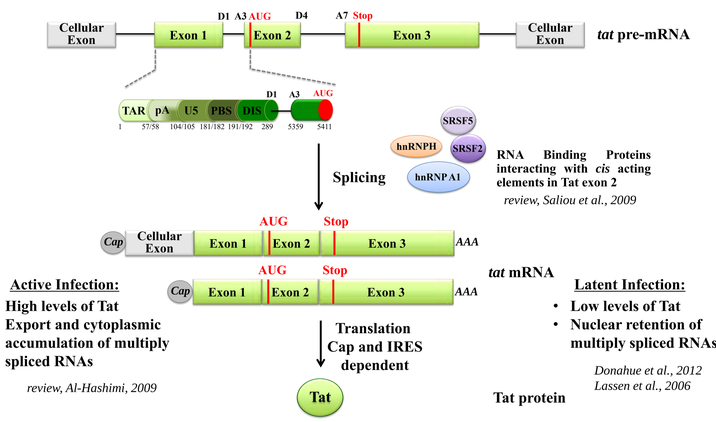


Figure 1. Tat protein production is highly regulated at the splicing and translational level. HIV latency arises when levels of the regulatory protein Tat fall below threshold levels. Due to it's central role in transcriptional transactivation and production of full-length viral mRNAs, limiting Tat, either because little mRNA is produced, or because it is not being translocated to the cytoplasm to be translated, contributes to the cells remaining latently infected.

Materials and methods

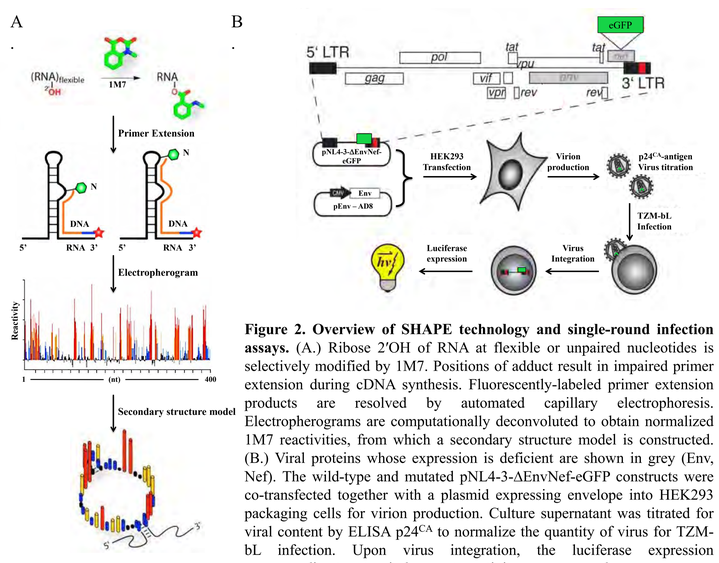
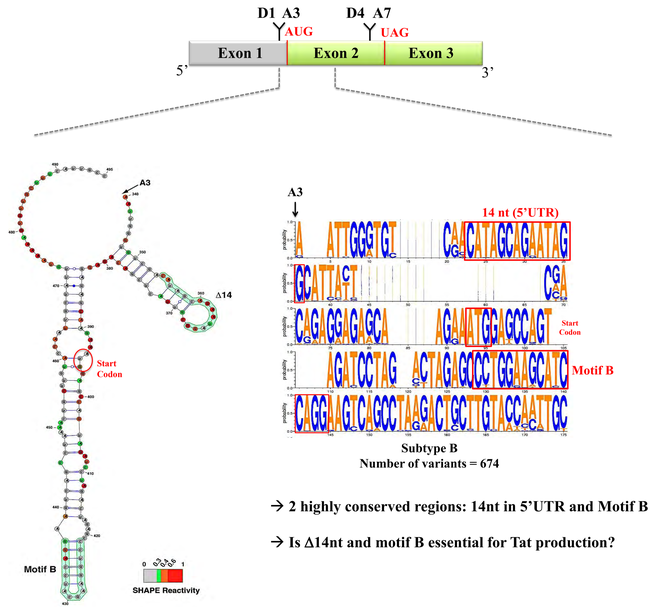


Figure 2. Overview of SHAPE technology and single-round infection assays. (A.) Ribose 2'OH of RNA at flexible or unpaired nucleotides is selectively modified by 1M7. Positions of adduct result in impaired primer extension during cDNA synthesis. Fluorescently-labeled primer extension products are resolved by automated capillary electrophoresis. Electropherograms are computationally deconvoluted to obtain normalized 1M7 reactivities, from which a secondary structure model is constructed. (B.) Viral proteins whose expression is deficient are shown in grey (Env, Nef). The wild-type and mutated pNL4-3-ΔEnvNef-eGFP constructs were co-transfected together with a plasmid expressing envelope into HEK293 packaging cells for virion production. Culture supernatant was titrated for viral content by ELISA $p24^{CA}$ to normalize the quantity of virus for TZM-bL infection. Upon virus integration, the luciferase expression corresponding to retroviral promoter activity was measured.

Results

➢ **Tat exon 2 harbours an IRES element that folds into a stable stem-loop structure.**



→ **2 highly conserved regions: 14nt in 5'UTR and Motif B**

→ **Is Δ14nt and motif B essential for Tat production?**

Figure 3. 2D structure analysis of *tat* mRNA by SHAPE. RNA structure analysis by chemical probing (1M7) revealed that Tat exon 2, which harbors an IRES element, folds into a stable stem-loop structure independently of the 5'UTR region. Sequence alignment of Tat-exon2 (nt 5778-6045, HXB2) from 2233 HIV strains using HIV Database (Los Alamos National Laboratory) revealed highly conserved sequences near the Tat start-codon.

➢ **Importance of motif B for Tat translation by an IRES mechanism and virus production.**

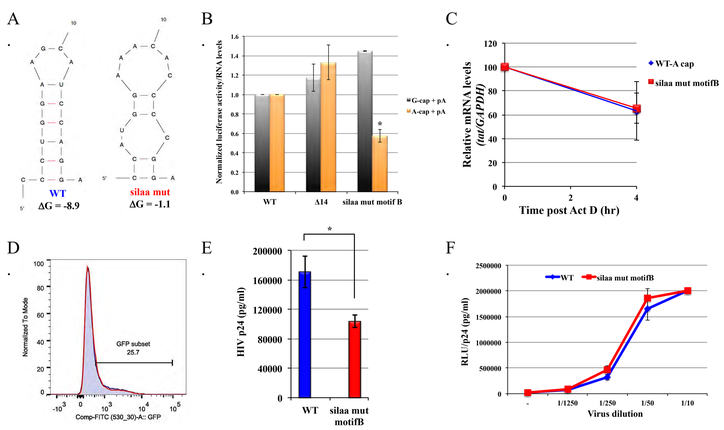
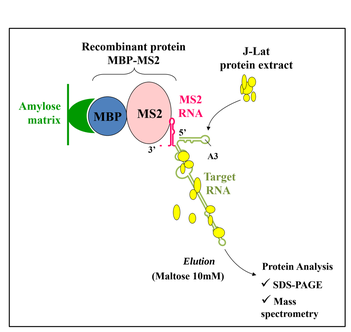


Figure 4. Effect of silent point mutations in motif B on Tat translation and virus production. Silent point mutations that disrupt *tat* mRNA structure (A.) induce a significant reduction in *tat* translation mediated by an IRES mechanism following RNA transfection of G- or A-capped and polyadenylated RNAs in TZM-bL cells followed by luminescence assay (B.). RNA stability was assessed by treatment with actinomycin D (5 µg/ml) for 0 and 4 h, followed by *tat* and GAPDH mRNAs quantification by RT-qPCR (C.). Flow cytometry analysis of transfected HEK293 cells with pNL4-3-DenvNef-eGFP wild-type or mutant and pEnv-Ad8 constructs (D.). Virion production was measured by quantifying the p24 viral capsid protein present in the supernatant of each transfection 72 h posttransfection by ELISA (E.). Viral infectivity assay: TZM-bl cells were infected with equal amounts of viral particles as determined by p24 ELISA. Luciferase activity (RLU) was measured 48 h after infection (F.).

➢ **Identification of new binding partners of *tat* mRNA by MBP-MS2 purification approach.**



Pull down method used to identify partners of *tat* mRNA from a T cell line model of latent infection.

✧ **RNA tested:**



✧ **J-Lat Protein Extract:**

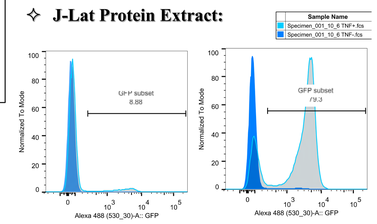
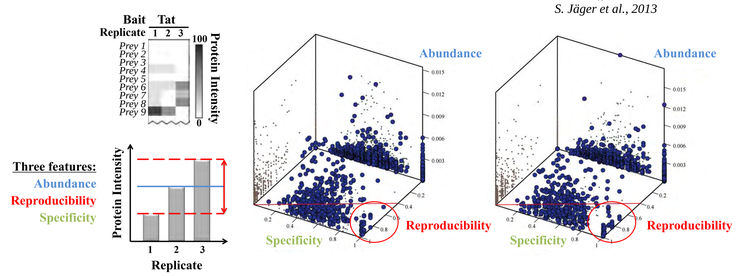
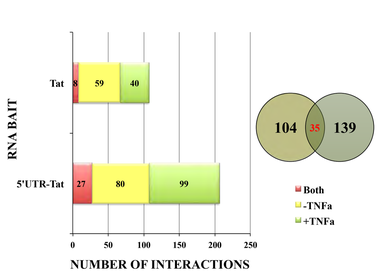


Figure 5. MBP-MS2 RNP purification approach. Representation of the fusion RNAs used in the pull down experiment with protein T cell line extract of latent infection (J-Lat clone 6.3 and 10.6). Retention of RNA containing three MS2 coat protein binding sites (MS2) on amylose beads was mediated by the MS2/MBP fusion protein. Analysis of the RNP complexes formed was achieved by 1D SDS-PAGE and mass spectrometry analysis.

➢ **243 new binding partners of *tat* mRNA were identified using a normalized label-free quantitative method combining 3 features (abundance, reproducibility and specificity).**

NM. Griffin et al., 2010
S. Jäger et al., 2013





	-TNFa	+TNFa
Viral	0	0
Transcription	6	6
mRNA Processing	18	23
EJC/mRNP	1	0
Transport	0	2
Translation	5	11
Exosome	0	1
Cleavage Stimulation Factors (CSTF)	0	0
Cleavage and Polyadenylation Specificity Factors (CPSF)	0	0
Chromatin DNA Binding/Repair/Replication	5	9
Chaperonin/Heat Shock	0	3
Proteosome	0	2
Cell Cycle Regulation	1	1
ER Function	1	3
Golgi Appartus	0	0
Mitochondrial Function	8	7
tRNA Function	3	2
Ubiquitination	3	2
Apoptotic	1	4
Others	55	71

Conclusions

Our study revealed a *cis*-acting RNA element underlying Tat codons recruits specific cellular proteins and, together they are important for Tat protein expression. The importance of the HIV Tat IRES RNA-binding proteins will be confirmed by siRNA knockdown, and assessed by their ability to induce cytoplasmic accumulation of HIV-1 RNAs in resting CD4+ T cells and release of replication-competent virus, while preserving a resting cellular phenotype. Therapeutic strategies targeting these proteins may constitute a novel approach towards reactivating HIV from latency and achieving a functional cure from residual HIV-1