Impact of HIV-1 TAR Sequence Diversity on its RNA Secondary Structure in HIV-1 DNA Genomes

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BACKGROUND

HIV-1 Trans-Activation Response element (TAR) is an RNA secondary structure located at the 5' end of the viral transcript. Binding of TAR and HIV-1 Tat protein promotes viral transcript elongation, therefore has a key role in virologic rebound in the absence of antiretroviral therapy.

Study hypothesis. **We hypothesize that TAR secondary structure and stability significantly differ across viral subtypes and across intact versus defective HIV-1 DNA genomes.**

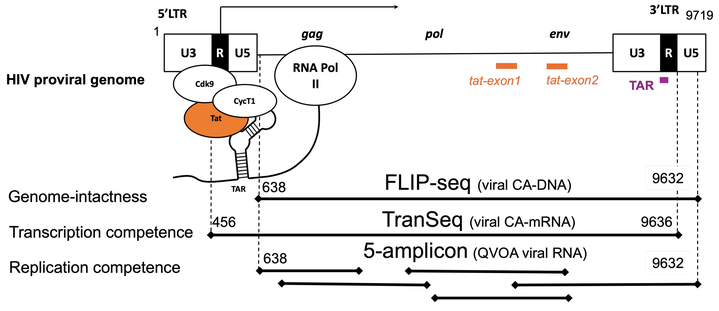
Importance. Our findings will give mechanistic insights into differences in transcriptional control across HIV-1 subtypes.

METHODS

Table 1. Study Groups

Source of TAR sequences	Count of TAR sequences	Number of Donors	HIV-1 Subtypes (number of donors)
Genome-intact viral DNA*	2230	46	A1 (4), B (14), C (5), D (9), AE (2), Recombinants (12)
Transcription competent viral mRNA	166	6	B (6)
Replication competent viral genomes	25	21	A1 (5), B (2), D (8), Recombinants (6)

Figure 1. In this study, we compared TARs associated with viral genomes in clinical samples at three functional levels: Near full-length proviral DNA genomes through DNA sequencing, viral mRNA transcripts, and replication competent viral genomes derived from quantitative viral outgrowth assays (QVOA). CA; cell-associated.

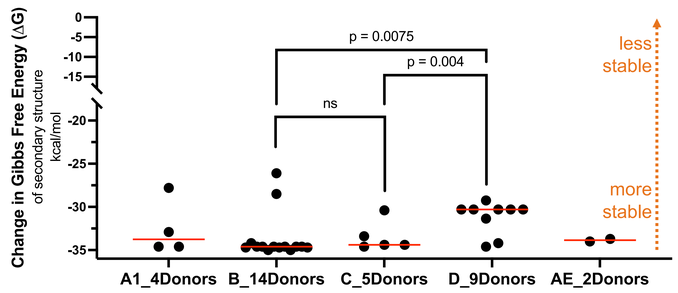


- Each viral DNA genome was classified as intact or defective using software **HIVSeqinR**.
- TAR secondary structure and its stability as measured by Gibbs free energy (ΔG) values were inferred using **Quikfold**; a **higher ΔG** is associated with **decreased** TAR **stability**.

RESULTS

In the HIV-1 DNA genome analysis, among the 46 study donors (2230 TAR sequences), TAR nucleotide diversity associated with intact viral genomes varied up to 13% interhost and 3% intrahost.

Figure 2. Subtype D TAR had the least stable TAR relative to subtypes B and C (p<0.008). Each dot represents one donor. Median Change Gibbs free energy ΔG values associated with CA-DNA TAR are plotted against other subtypes. The median number of viral DNA genomes associated with each dot is 21.5 (IQR: 16-49.25).



TAR associated with Genome Intact and Defective Proviral Genomes (CA-DNA)

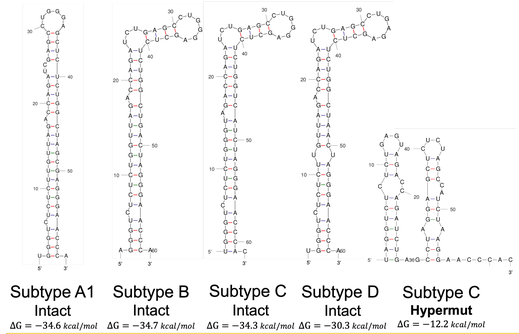
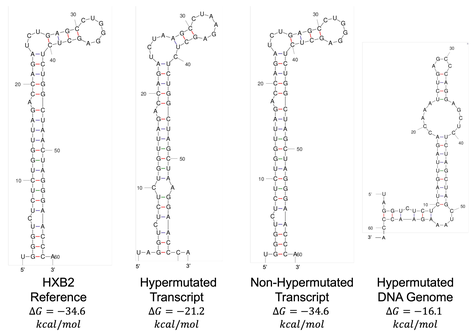


Figure 3. HIV-1 subtypes have distinct TAR secondary structures. Defective genomes showed abnormal structures with key features such as the lack of a 3-nucleotide pyrimidine bulge (Tat-TAR interaction residues) and a hexaloop. In contrast, TAR associated with subtype A1 is mostly associated with a 2-nucleotide bulge.

TAR associated with Transcriptionally Active Viruses (CA-mRNA)

Figure 4. In our analysis of *ex vivo* viral mRNA transcripts, we observed that transcripts associated with APOBEC-3G/3F hypermutations were able to actively transcribe. Despite having lower stability (p<0.0001), TAR sequences associated with these transcriptionally-active but hypermutated viral genomes maintained a similar structure relative to TAR sequences associated with replication competent genomes (see Figure 5). The UCU bulges observed in subtype B TARs were preserved in all these transcripts.



TAR associated with Replicon Competent Viruses (QVOA culture supernatant)

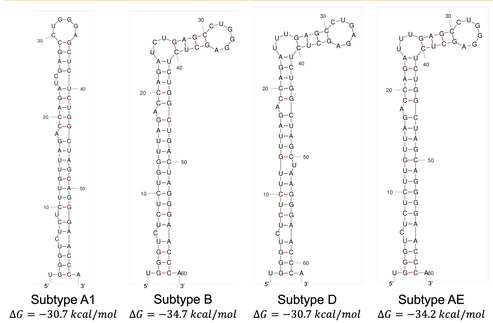
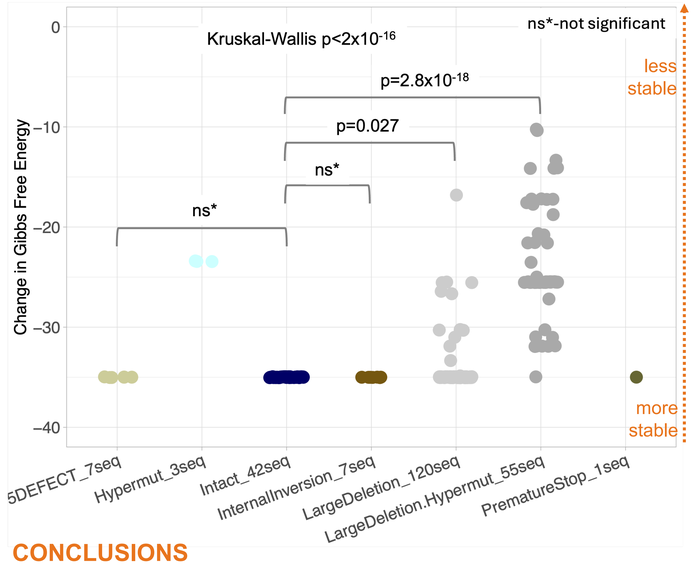


Figure 5. In our analysis of TAR associated with replication competent viruses derived from QVOA culture supernatant, we observed TAR secondary structures similar to those associated with genome-intact CA-DNA (see Figure 3). Again, subtype A1 was associated with a 2-nucleotide bulge whereas B, D were mostly associated with a 3-nucleotide bulge.

Figure 6. HIV-1 CA-DNA genomes frequently contained gross defects. This figure shows that within a single donor, TAR secondary structure stability was **not significantly different** between intact and defective genomes, except in genomes containing large deletions and/or APOBEC 3G/3F-associated hypermutations. In other words, we observed no evidence of selection for specific TAR genotypes in the intact versus defective compartments except when a genome was subjected to hypermutation.

Representative Donor OM5267



CONCLUSIONS

- Our study reveals HIV-1 TAR genotypes are not identical across viral subtypes
- There is no significant difference in TAR stability between genome-intact versus defective viruses, except for TARs associated with hypermutated genomes.
- Differences in TAR stability may imply differences in the extent of viral transcription activity.
- Future studies should examine whether TAR stability can predict the quantity of viral transcripts produced.

ADDITIONAL KEY INFORMATION

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