Investigating Competition between the HIV-1 Proteins Rev and Gag for Stem 1 of the Rev Response Element

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Abstract

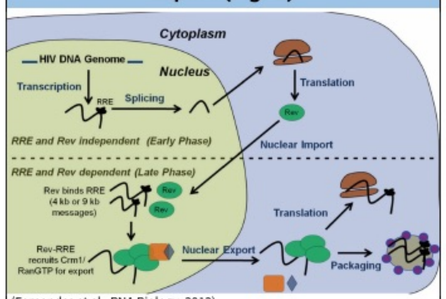
HIV replication requires the export of full length RNA genome transcripts from the nucleus to the cytoplasm, where the genome is packaged. Host surveillance mechanisms prevent such RNA export, so HIV uses Rev, a viral protein translated from fully spliced transcripts to enter the nucleus and bind to a highly conserved RNA element of the HIV genome, the Rev Response Element (RRE), which is retained on un/incompletely spliced RNA. The Rev-RRE complex binds to host export machinery, and exits the nucleus to the cytoplasm, where unspliced RNA is available for translation or packaging. Rev binds to the RRE on two stem II binding sites and on one purine-rich bulge in stem 1. The RRE has been shown to also be bound by Gag –a viral protein involved in genome packaging– at the same RRE stem I binding region as Rev. To understand the interaction and biological relevance between Rev and Gag on RRE stem 1, we use a peptide containing the RNA-binding, arginine-rich motif (ARM) of Rev, a protein containing the nucleocapsid (NC) domain of Gag, and truncated RRE stem I fragments in EMSA and ITC studies. Some RNA fragments include mutations that allow us to probe for specific protein binding sites. We find that NC displays tighter binding affinity to our stem 1 constructs than the Rev ARM peptide, which may suggest that Gag binds to the RRE in the cytoplasm and displaces Rev from the stem 1 binding site. This interaction may be biologically relevant and may represent a link between nuclear export of the genome and subsequent genome packaging. Future studies are needed to more accurately explore this competitive interaction, such as the usage of full-length RNA constructs, which would contain more Rev binding sites, and full-length protein constructs that would more accurately represent physiological interactions.

Background

The HIV Pandemic

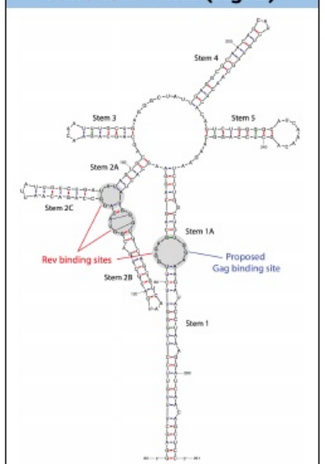
- HIV is an ongoing pandemic, with 37 million infections and no cure in 2021 (UNAIDS, 2021).
- Current treatments require continual use, cause harmful side effects, and may be inaccessible for many.
- The Rev Response Element (RRE), is a portion of the HIV RNA, that binds to Rev, a viral RNA-binding protein required for nuclear genome export. Both Rev and the RRE are highly conserved and may serve as future drug targets.

RRE and Rev Regulation of Nuclear Export (Fig. 1)

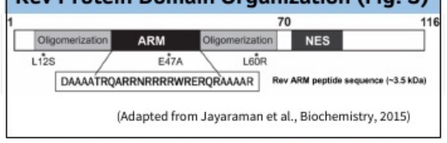


(Fernandes et al., RNA Biology, 2012)

Predicted Secondary Structure of Minimal Functional RRE (Fig. 2)

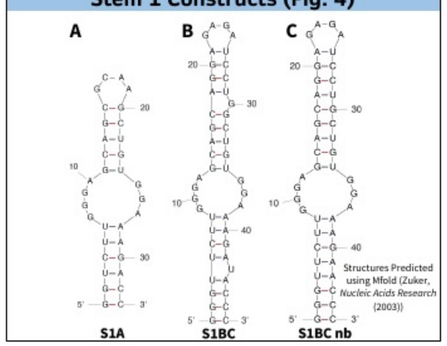


Rev Protein Domain Organization (Fig. 3)



(Adapted from Jayaraman et al., Biochemistry, 2015)

Predicted Secondary Structure of RRE Stem 1 Constructs (Fig. 4)



Methods

RNA Constructs

RRE-S1A: 33 nucleotides long; contains RRE stem 1A.
RRE-S1BC: 47 nucleotides long; longer stem 1 construct containing RRE stem 1A
RRE-S1BCnb: Similar to S1BC, but lacking two smaller bulges above and below the larger purine-rich bulge.

Protein Constructs

NC: Nucleocapsid domain of the viral structural protein Gag, which has been shown to bind to the RRE (Kutluay et al., Cell, 2014)
Rev Peptide: 27 amino acids long; contains the Rev Arginine Rich Motif (ARM)

Sample Preparation

- All samples in 1x PI Buffer: 2mM Tris-Base, 140mM KCl, 10mM NaCl, .5mM $MgCl_2$, 0.5mM TCEP
- EMSA Samples were run on 16% Native, Tris Base and Tris Boric Acid Polyacrylamide Gels for 1hr at 220V.
- ITC Samples dialyzed overnight at 4°C, and experiments were conducted at 25 °C, with 19 or 30 injections.

Electrophoretic Mobility Shift Assay (EMSA)

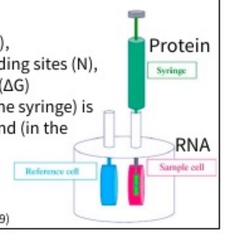
- An electric current moves charged items through a gel matrix according to charge and size, with larger items moving slower
- Gels are usually made of agarose or polyacrylamide
- Our EMSA studies consist of RNA-protein titrations

Isothermal Titration Calorimetry (ITC) (Fig. 5)

ITC measures:
- enthalpy (ΔH),
- binding affinity (K_D),
- The number of binding sites (N),
- Gibb's free energy (ΔG)

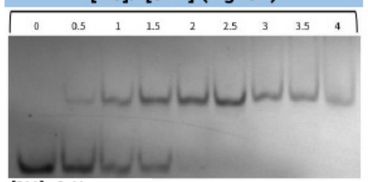
when the titrant (in the syringe) is injected into the titrand (in the cell).

(Adapted from Srivastava & Yadav, Data Processing Handbook for Complex Biological Data Sources, 2019)



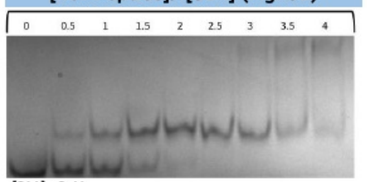
EMSA Results (Fig. 6)

[NC]/ [S1A] (Fig. 6A)



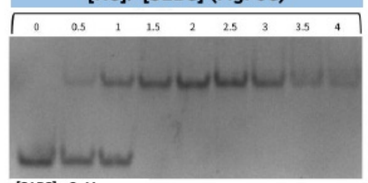
[S1A]= 2μM

[Rev Peptide]/ [S1A] (Fig. 6B)



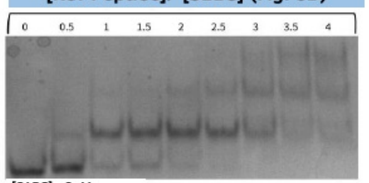
[S1A]= 2μM

[NC]/ [S1BC] (Fig. 6C)



[S1BC]= 2μM

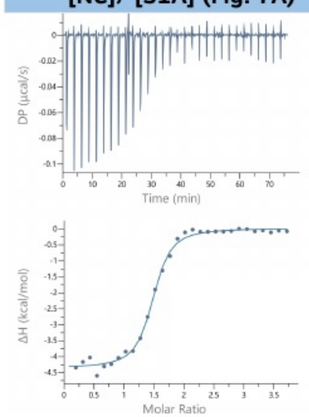
[Rev Peptide]/ [S1BC] (Fig. 6D)



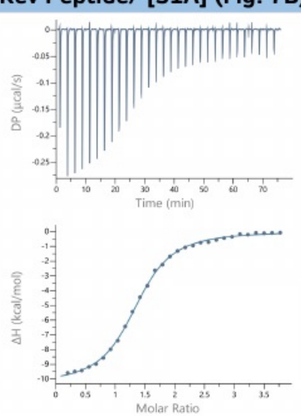
[S1BC]= 2μM

ITC Results: RRE-S1A (Fig. 7)

[NC]/ [S1A] (Fig. 7A)

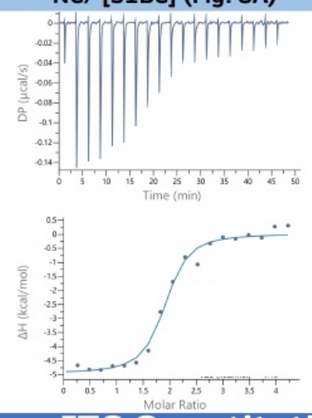


Rev Peptide/ [S1A] (Fig. 7B)

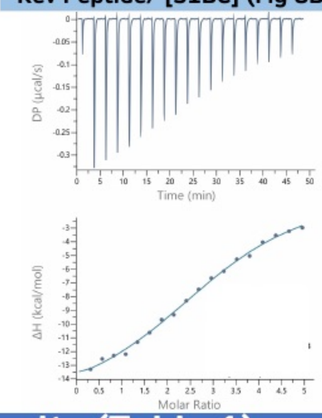


ITC Results: RRE-S1BC (Fig. 8)

NC/ [S1BC] (Fig. 8A)



Rev Peptide/ [S1BC] (Fig 8B)

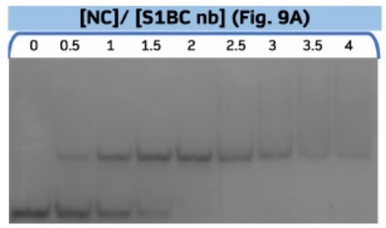


ITC Quantitative Results (Table 1)

Protein (Syringe)	RNA (Cell)	Binding Affinity (K_D, nM)	Number of Binding Sites (N)	Enthalpy Change (ΔH, kcal/mol)	Free Energy Change (ΔG, kcal/mol)
NC	S1A	150 ± 23.4	1.43 ± 0.012	-4.37 ± 0.074	-9.31
Rev Peptide	S1A	718 ± 35.9	1.35 ± 0.0069	-10.4 ± 0.10	-8.38
NC	S1BC	117 ± 30.8	1.83 ± 0.031	-4.98 ± 0.122	-9.46
Rev Peptide	S1BC	3160 ± 255	3.14 ± 0.04	-16.4 ± 0.374	-7.50

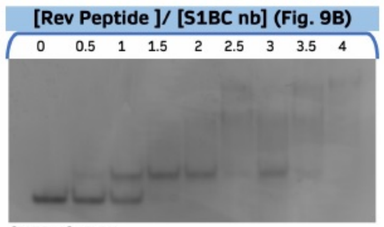
Preliminary S1BC nb EMSA Results (Fig. 9)

[NC]/ [S1BC nb] (Fig. 9A)



[S1BC nb]= 2μM

[Rev Peptide]/ [S1BC nb] (Fig. 9B)



[S1BC nb]= 2μM

Summary

- NC/RNA Saturation occurs at molar ratios of: 2 (S1A), 1.5 (S1BC), and 2 (S1BC nb), while Rev Peptide/ Saturation occurs at molar ratios of ~2.5 (S1A), ~3 (S1BC) and ~2 (S1BC nb).
- ITC studies show higher NC binding affinity to both S1A and S1BC RNAs (150 nM and 117 nM, respectively), compared to Rev Peptide across both RNAs (718 nM for S1A and 3160 nM for S1BC).
- NC may displace Rev after the genome is exported from the nucleus, but more data is required to test and fully support this hypothesis.

Future Directions

- ITC studies using S1BC nb can characterize whether the two smaller bulges serve as NC or Rev binding sites.
- NMR studies accurately characterize structures and interactions at an atomic level.

References

(1) UNAIDS, 2021 2) Fernandes et al., 2012; (3) Zuker, Nucleic Acids Research, 2003; (4) Jayaraman et al., Biochemistry, 2015 (5); Kutluay et al., Cell, 2014 (6); Srivastava & Yadav, Data Processing Handbook for Complex Biological Data Sources, 2019

Acknowledgements

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