In-Vivo Dose Response Curves of HIV Rev to Rev Response Element Using RBP-Scan

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INTRODUCTION

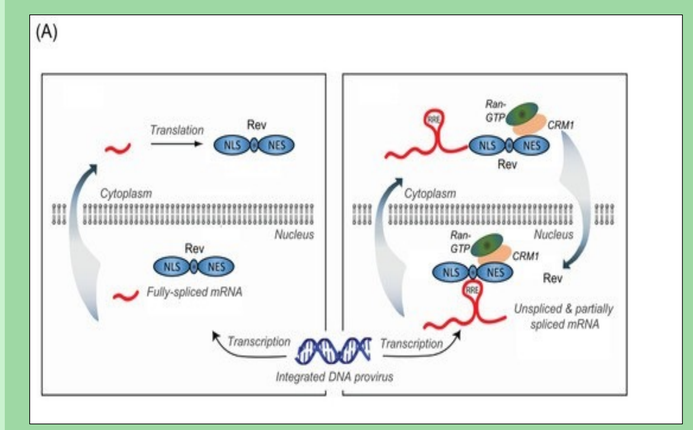

Figure 1. Rev binds to the RRE to export unspliced and partially spliced HIV mRNAs via CRM1/Ran-GTP, while fully spliced mRNAs are exported independently. Image acquired from Sherpa and Le Grice, *Viruses* (2020).

- **HIV replication depends on Rev**, a regulatory protein essential for the nuclear export of unspliced and singly spliced viral RNAs.
- Rev binds specifically to the **Rev Response Element (RRE)**, a highly structured RNA located within the env gene of HIV transcripts (Fig. 1)
- The **RRE contains multiple stem-loops**, with **Stem-Loop IIB (SLIIB)** acting as the **primary high-affinity binding site** where Rev first binds.
- The Rev-RRE complex interacts with **CRM1 (Exportin 1)** and **Ran-GTP**, which drive translocation through the nuclear pore (Fig. 2)
- Once bound at SLIIB, Rev **multimerizes cooperatively along the RRE**, forming an export-competent ribonucleoprotein complex.
- **Ran-GTP hydrolysis in the cytoplasm** triggers complex disassembly, releasing viral RNA for translation or packaging.
- **Understanding Rev-RRE binding cooperativity** is crucial for defining HIV post-transcriptional regulation and identifying **therapeutic targets** that disrupt viral RNA export.

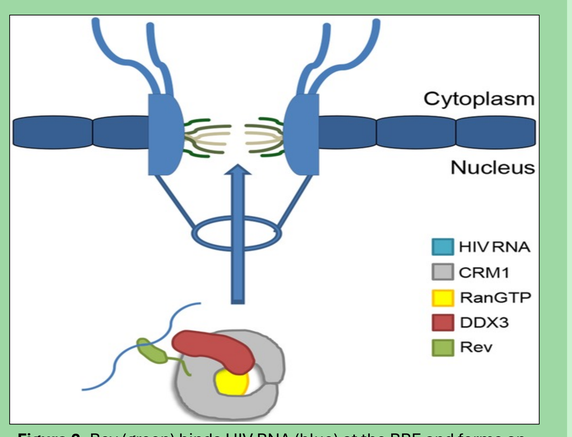

Figure 2. Rev (green) binds HIV RNA (blue) at the RRE and forms an export complex with CRM1 (gray) and RanGTP (yellow). DDX3 (red) associates with the complex to facilitate translocation through the nuclear pore into the cytoplasm. Image acquired from Mahboobi *et al.*, *PLoS ONE* (2015).

Figure 3. Stem Loop IIB (Red) interacting with Rev Minimum Subunit (Green).

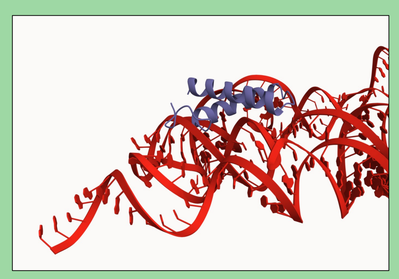

Figure 4. Full RRE (Red) interacting with Rev Minimum Subunit (Purple).

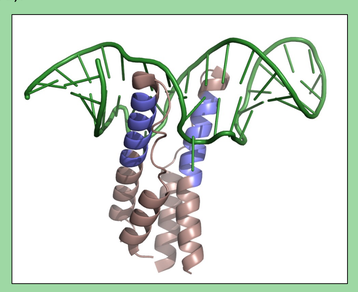

Figure 5. Full Rev (Tan and Purple) multimerization on Full RRE (Green).

METHODOLOGY

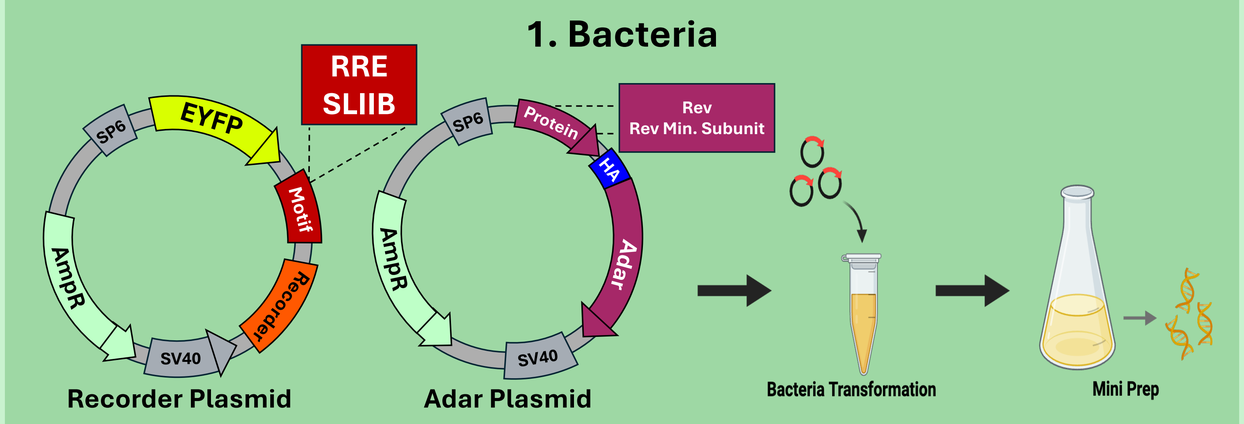

Figure 6. Methodology of an RBP-Scan A-to-G Edit Analysis. Images acquired from BioRender (2025) and Kretov *et al.* (2025).

DOSE RESPONSE CURVES

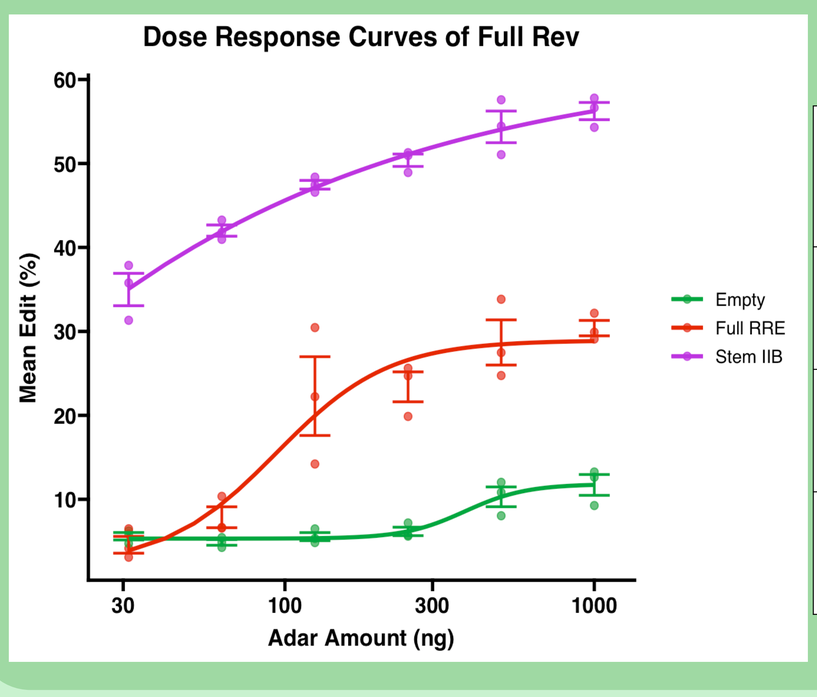

Figure 7. Dose Response Curves of Full Rev to an Empty Recorder (1313), Full RRE, and Stem Loop IIB. Error bars represent Standard Error of the Mean.

Group	Hill Coefficients
Empty (1313)	0.5
Full RRE	1.86
Stem IIB	0.84

Figure 8. Table of Hill Coefficients for each experimental group.

BLOT IMAGING

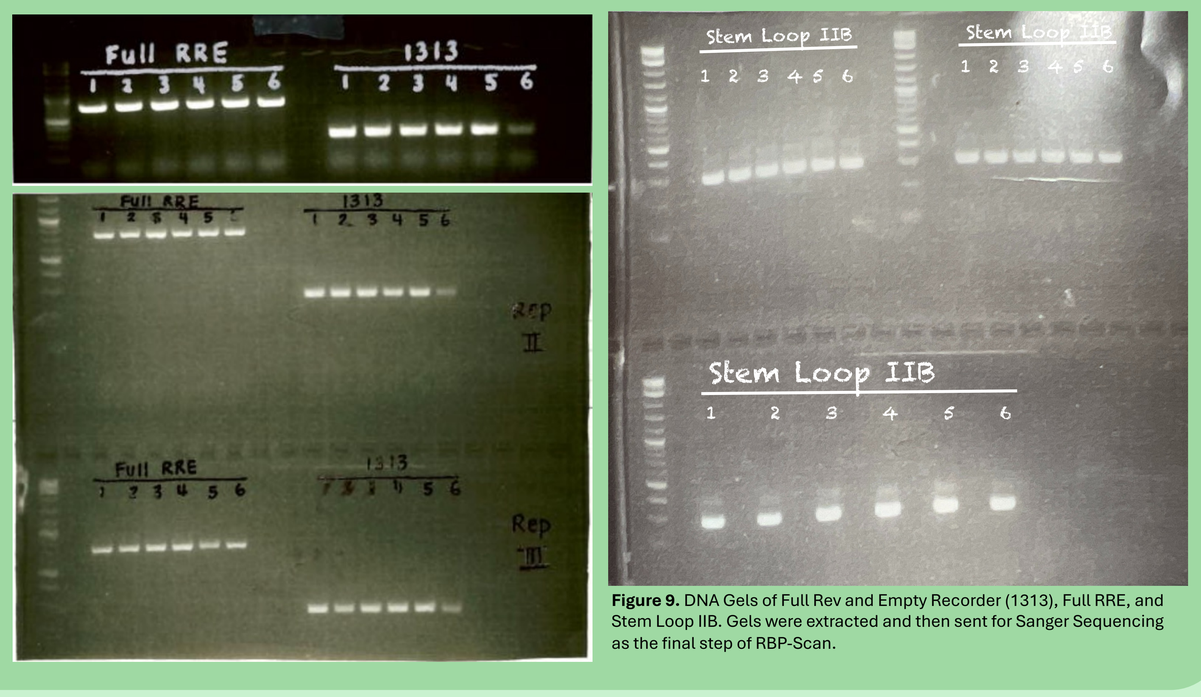

Figure 9. DNA Gels of Full Rev and Empty Recorder (1313), Full RRE, and Stem Loop IIB. Gels were extracted and then sent for Sanger Sequencing as the final step of RBP-Scan.

DISCUSSIONS

- This study demonstrates that **cooperative Rev binding** occurs only in the context of the **full RRE structure**, not the isolated SLIIB domain. The full RRE's Hill coefficient (1.86) reflects multimerization behavior, while SLIIB's coefficient (0.84) confirms its role as an initial high-affinity contact without enabling further assembly (Fig. 10).

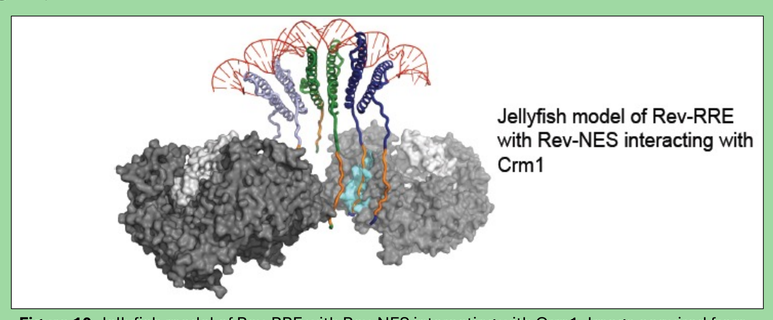

Figure 10. Jellyfish model of Rev-RRE with Rev-NES interacting with Crm1. Image acquired from Jdf2001 (2025).

- Quantifying Hill coefficients in a live-cell system provides a **functional readout of RNA–protein interaction dynamics**, supporting broader applications of this approach in antiviral drug development and RNA biology research.
- By quantifying cooperativity and K_d using ***in-vivo*** RNA editing data, we establish a framework for analyzing **RNA–protein interactions** under physiological conditions.
- **Future directions:**
  - Design and screen **small molecules or peptides** that bind to SLIIB and block Rev recognition.
  - Use **cell-based reporter assays** to evaluate how these inhibitors affect RNA export and HIV replication.
  - Explore **structure-guided mutagenesis** to pinpoint residues essential for cooperative binding.
- These approaches may contribute to **novel therapeutic strategies** targeting HIV post-transcriptional regulation.

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