UNPAIRED GUANOSINES IN HIV-2 LEADER RNA DIRECT HIV-2 GENOME PACKAGING

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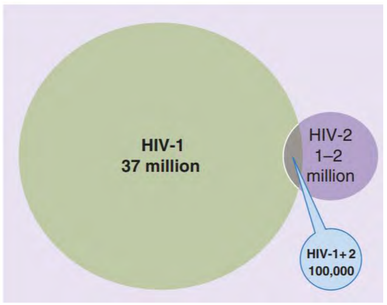
INTRODUCTION

Worldwide Prevalence of HIV-1 & HIV-2



UNAIDS, Nov 2016

Human immunodeficiency viruses types I and II (HIV-1 and HIV-2) resulted from cross-species transmission of simian lentiviruses into humans:

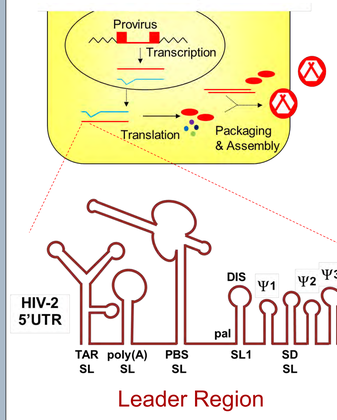
- HIV-1 is derived from SIV_{cpz} and SIV_{gor}
- HIV-2 is derived from SIV_{smm}

Both HIVs cause AIDS but HIV-2 is less prevalent and less pathogenic

The potential for recombination in co-infected individuals and limited cross-reactivity of HIV-1 antiretrovirals on HIV-2 compels increased HIV-2 research

RESEARCH GOAL

HIV Gene Expression & RNA Packaging



Leader Region

- To generate infectious particles, HIV packages two copies of full-length viral RNA
- Interactions between HIV Gag and *cis*-acting elements in the leader region mediate genome packaging
- C*is*-acting RNA packaging elements are not well defined in HIV-2

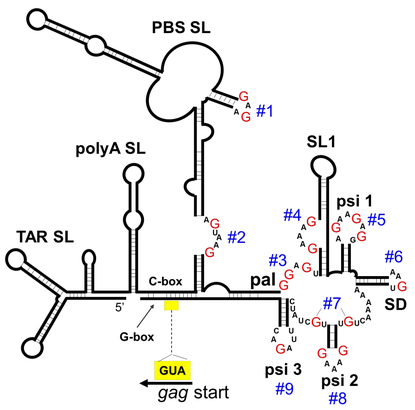
GOAL: Identify c*is*-acting elements important for HIV-2 RNA packaging

HYPOTHESIS & APPROACH

Unpaired guanosines have been shown to be important in HIV-1 and murine leukemia virus RNA packaging. Based on these observations, we hypothesized that unpaired guanosines in the HIV-2 leader RNA are important elements for packaging

Approach

Selected 9 predicted sites of unpaired guanosines for G>A Substitutions

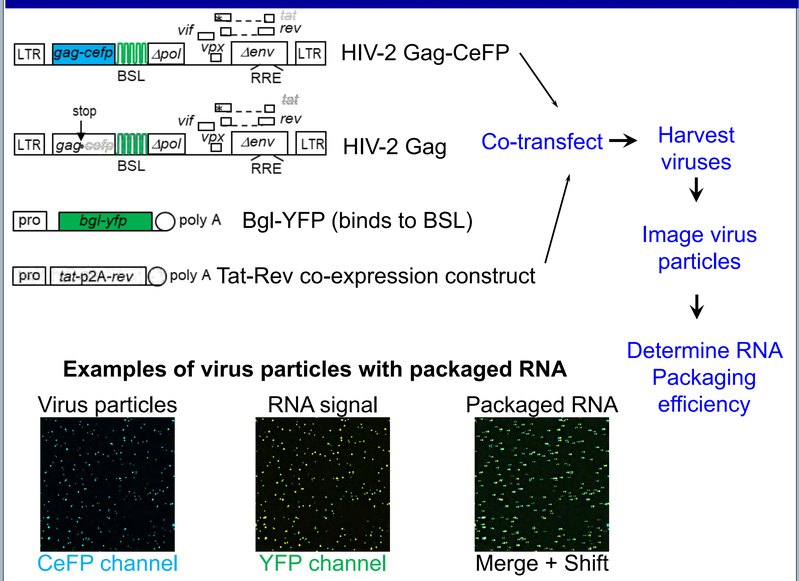


HIV-2 leader RNA *in vitro* SHAPE Structure;
Modified from Purzycka *et al.*, *Nucleic Acids Res*, 2011.

Based on the HIV-2 leader RNA structure from chemical probing studies, unpaired guanosines were selected and mutated

Determine effects of guanosine mutations on HIV-2 RNA packaging

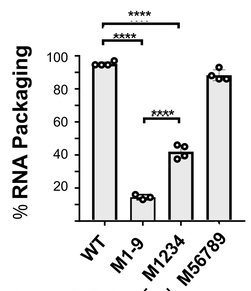
EXPERIMENTAL SYSTEM – VISUALIZING SINGLE VIRIONS



Examples of virus particles with packaged RNA

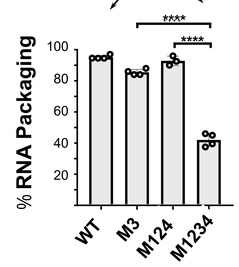
% RNA Packaging: Proportion of virus particles with RNA signal

RESULTS I

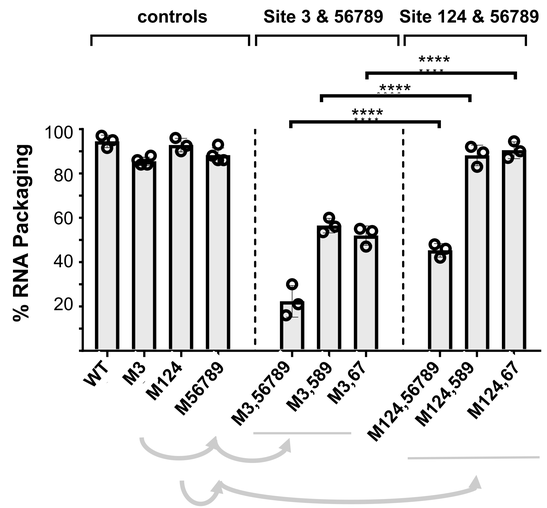


- Substituting guanosines in 9 sites severely reduces RNA packaging
- Sites 1-4 contain elements important for RNA packaging

Separate sites 1,2,3,4



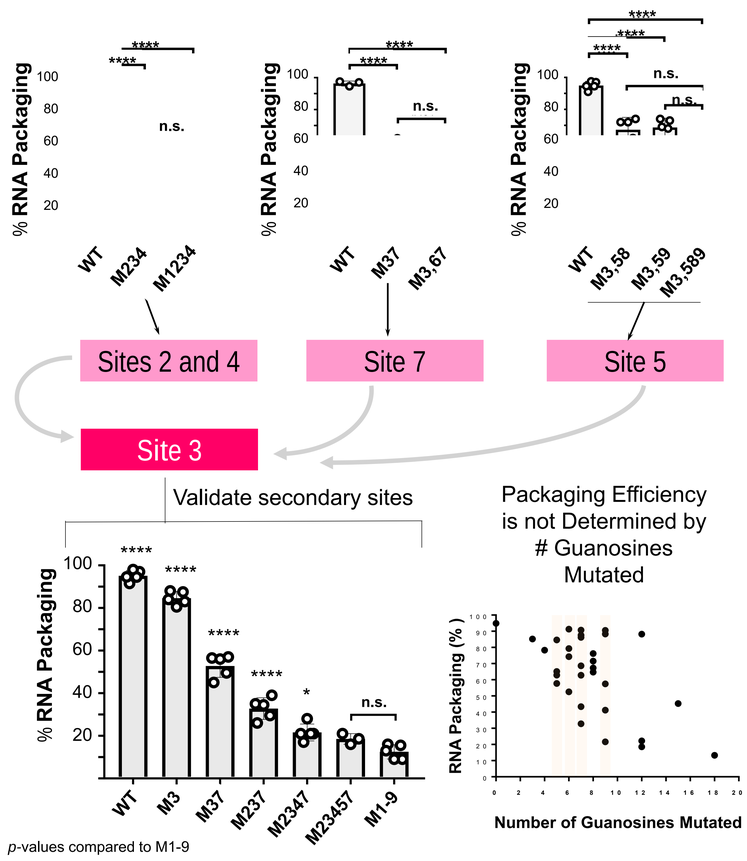
- No single site is responsible for RNA packaging
- Data suggests additive or synergistic effects between sites



- Packaging is an effect of synergy between multiple sites
- Site 3 exerts the most dominant synergistic effects

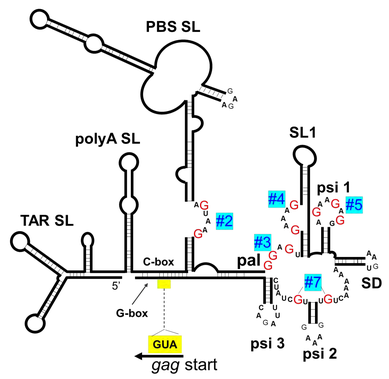
RESULTS II

Identification of Specific Sites that Coordinate with Site 3



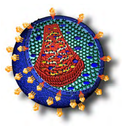
p-values compared to M1-9

C*is*-acting elements important for HIV-2 RNA Packaging



SUMMARY

- Using single virion analysis we have identified *cis*-acting elements in HIV-2 leader RNA that are important for efficient packaging of the viral genome
- We identified a primary packaging determinant and several secondary sites
- Our data suggest that combination of primary and secondary guanosine sites in the leader RNA drive efficient packaging of the viral genome
- Currently investigating how these guanosine elements direct RNA packaging



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