Determining How Stem-loop Structure Thermodynamic Stability Influences Frameshift Efficiency at the HTLV-1 *gag-pro* Frameshift Site

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Background:

The human T-lymphotropic virus type 1 (HTLV-1) RNA genome includes two programmed -1 ribosomal frameshift (-1 PRF) sites. These sites allow ribosomes access to alternate reading frames encoding critical viral enzymes. The *gag-pro* frameshift site includes a slippery sequence, spacer, and stem-loop structure. How the stem-loop acts to promote frameshifting is unclear. Previous HTLV-2 research showed that changes to the *gag-pro* frameshift site stem-loop thermodynamic stability influenced its frameshift efficiency to a modest degree. There is substantial conservation between the HTLV-1 and HTLV-2 *gag-pro* frameshift site sequences (86%) and structures. We hypothesized that the HTLV-1 *gag-pro* frameshift efficiency would be similarly influenced by its stem-loop thermodynamic stability.

Methods:

To test this hypothesis, we designed 15 stem-loop mutants (SLMs) with varied base-pair composition. These mutations decoupled changes in overall thermodynamic stability from those localized to the stem-loop base. The SLM thermodynamic stabilities were calculated using nearest neighbor parameters and the *in vitro* frameshift efficiencies were measured with a dual-luciferase assay. Correlations between frameshift efficiency and thermodynamic stability were subsequently assessed.

Results:

Preliminarily results reveal a moderate correlation between the SLM stem-loop overall thermodynamic stability and frameshifting efficiency. No correlation was observed between the thermodynamic stability of the stem-loop base and frameshifting efficiency.

Conclusion:

While the overall thermodynamic stability does impact the frameshift efficiency, it cannot be used exclusively to predict it. This reflects a complex interplay between the frameshift site elements. Overall, our preliminary results suggest a conserved function for the *gag-pro* frameshift site stem-loop between the HTLV-1 and HTLV-2 retroviruses.

Disclosure of interest statement:

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