Investigating the Role of Base-Triples in the HTLV-1 *pro-pol* Frameshift Site Pseudoknot

Authors:
Mouzakis KD[1], Maille MM[1]

[1] Department of Chemistry and Biochemistry, Loyola Marymount University, Los Angeles, CA, United States of America

Background:
The human T-cell leukemia virus type 1 (HTLV-1) RNA genome includes two programmed -1 ribosomal frameshift sites. Each site includes three RNA elements: a slippery sequence, a spacer, and a structure. We recently determined that the pro-pol frameshift site contains a pseudoknot structure. Research on other viral frameshift site pseudoknots indicates that contacts between single-stranded loop nucleotides and base-pairs in the stems are often critical to pseudoknot function. Although the HTLV-1 pseudoknot tertiary structure is not determined, base-triples may form between its two loops and two stems.

Methods:
To test the hypothesis that these tertiary contacts were critical to its function, the frameshift efficiencies were measured for the wild-type frameshift site and three mutants. Two mutants (A2295U and Loop 2 A to U) were designed to disrupt putative base-triples forming between loop 2 and stem 1. A third mutant (U2272C) was designed to evaluate the impact of a stem 2 G•U to G-C substitution.

Results:
Preliminary data from quadruplicate measurements showed that the frameshift efficiency for all three mutants was moderately increased relative to the wild-type frameshift site.

Conclusion:
Whether the changes in the A2295U and Loop 2 A to U mutant frameshift efficiencies were caused by changes to tertiary contacts is hard to determine without complementary methods that establish their presence. Although the increase in the U2272C mutant frameshift efficiency could relate to tertiary contacts, it is more easily explained by a change to the stem 2 thermodynamic stability. Future work will focus on leveraging other methods to evaluate these possibilities.

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