ABSTRACT

TITLE:	Determining the Secondary Structure HTLV Pro-Pol Frameshift Siten Using NMR and Native Gel Analysis
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PRESENTED AT:	2014 Undergraduate Research and Creative Activities Symposium, Fort Lewis College

ABSTRACT: Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus that targets CD+ T-cells in humans. Within its RNA genome, it has two open reading frames that are only accessed through programmed ribosomal frameshifting (PRF). PRF is defined by a directed change to the ribosome's reading frame during the process of translation. Each PRF sites consist of two RNA elements: a heptanucleotide slippery sequence followed by a downstream RNA structure. In retroviral frameshift sites, this RNA structure is typically a pseudoknot. The frequency of frameshifting is specific to the frameshift site. Additionally, this frequency is important for maintaining appropriate molar ratios of proteins translated from the original and alternative reading frames. HTLV-1's second frameshift site is located within the pro and pol overlap. This site contains a slippery sequence, spacer region, and a RNA structure. The RNA structure is predicted to be a pseudoknot. Pseudoknot structures occur when nucleotides in the loop segment of a stem loop base-pair with nucleotides downstream from the base of the stem. The goals of this work were to use nuclear magnetic resonance (NMR) and native gel analysis to determine the pro-pol frameshift site RNA structure. In addition to the wild-type RNA, three other constructs were used to evaluate the secondary structure and folding of the predicted pseudoknot. Two of these constructs have mutations in the loop and downstream nucleotides that eliminate base-pairing in the pseudoknot formation. Currently, the stem loop RNA has been purified and its purity and folding examined. Additionally, template and non-template sequences for the three pseudoknot RNAs (WT, PKM1, and PKM2) were cloned into the Puc19 plasmid and transformed into E. coli.