ABSTRACT

TITLE:	Determining the Secondary Structure of the HTLV Pro-Pol Frameshift Site Using Shape Chemical Probing.
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ABSTRACT: Human T-cell Leukemia virus (HTLV) is a retrovirus that infects adult CD4+ T-cells. Production of infectious virions is dependent on two programmed changes in reading frame during translation. These changes are triggered by specific sequences and structures within the HTLV ribonucleic acid (RNA) genome. In the context of retroviruses, frameshiting is frequently used to initiate the translation of viral enzymatic proteins. HTLV's second frameshift site is positioned within the overlap of the pro and pol open reading frames. This site contains two RNA elements: a slippery sequence, UUUAAAC, and a predicted pseudoknot structure. Pseudoknot's are composed of stem-loops that have their loop nucleotides base-paired to downstream RNA. To date, no experimental evidence has confirmed the stable formation of the HTLV pro-pol frameshift site pseudoknot structure. The goal of this work is to use chemical probing methods to determine the pro-pol frameshift site RNA structure. Specifically, selective 2'-hydroxyl acylation and primer extension (SHAPE) will be used to identify areas of base-pairing within the frameshift site RNA. During these experiments, the N-methyl-isatoic anhydride (NMIA) chemical does not acylate nucleotides involved in stable base-paring interactions. Therefore, the pattern of RNA modification will reveal which nucleotides within an RNA are base-paired or single-stranded. Computational programs can be paired with SHAPE data to predict the secondary structure.