ABSTRACT

TITLE:	Determination of the Secondary RNA Structure and Its Importance to the HTLV-1 Pro-Pol Frameshift Site
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ABSTRACT: Ribonucleic acids (RNAs) are a group of macromolecules that encode genetic information. Primary RNA structures can fold into various complex secondary structures, which are important for numerous cellular functions. Many single-stranded RNAs serve as the genetic material for several viruses, such as the Human T-Cell Lymphotrophic Virus Type I (HTLV-1). Five to ten percent of the time, HTLV-1 infections result in leukemia. HTLV-1 is a retrovirus that uses cis-acting regulatory elements in RNA to prompt a frameshift during viral mRNA translation, allowing for the translation of genes in alternative reading frames that are critical to viral infectivity. The pro-pol frameshift site is the second frameshift site in the virus' genome and is defined by two signals: a slippery sequence and an RNA structure downstream of the slippery site. In order to target HTLV-1, it is important to experimentally determine RNA structures that contribute to its pathogenic characteristics. The RNA structure downstream of the slippery site is proposed to be a pseudoknot (a structural motif formed when the nucleotides in the loop of a stem-loop base-pair with nucleotides downstream of the structure). So far no structural evidence supporting or refuting this structure has been published. The goal of my work is to determine the RNA structure in the HTLV-1 pro-pol frameshift site. Thus far, I have designed RNA constructs containing the HTLV-1 frameshift site, predicted their structures, and successfully cloned the DNA that codes for the RNAs into a puc19 vector. I have also transcribed and purified one of these RNAs. Future experiments will include chemical modification of the RNAs using selective 2-hydroxyl acylation analyzed by primer extension (SHAPE) to determine which regions of the RNA structure are base-paired and which regions are not and secondary RNA structure prediction using SHAPE data and various computer programs.