ABSTRACT

TITLE:	Minimal RNA Sequence Requirement of the HTLV-1 Gag-Pro RNA Frameshift Site
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PRESENTED AT:	2016 Undergraduate Research and Creative Activities Symposium, Fort Lewis College

ABSTRACT: Human T-cell leukemia Virus Type I (HTLV-1) was the first identified human retrovirus, identified in 1980 (1). Infection with HTLV-1 results in adult T-cell leukemia with 5-10% incidence. An estimated 15-20 million individuals worldwide are infected with HTLV-1 (2). Replication of retroviruses, such as HTLV, is dependent upon synthesis of viral structural and enzymatic proteins. Synthesis of HTLV's enzymatic proteins is dependent upon two -1 programmed ribosomal frameshift (PRF) events. PRF is defined as a programmed change in the ribosome's reading frame during translation. During a -1 PRF, the ribosome shifts one nucleotide in the 5' direction along the RNA when it is positioned on a "slippery sequence." Slippery sequences have a consensus sequence of XXXYYYZ, where X can be any nucleotide, Y can be A or U, and Z cannot be G. This sequence of nucleotides allows favorable tRNA anticodon:codon base-pairing between the tRNAs and mRNA in both the original (0) or alternate (-1) reading frames. In this work, the HTLV-1 gag-pro -1 frameshift site requirements are investigated. The gag-pro frameshift site consists of a heptanucleotide slippery sequence (AAAAAAC), followed by a downstream structure. A stem-loop structure is predicted downstream of the slippery sequence (3). We are investigating the minimal RNA sequence required for frameshifting. To determine the minimal sequence requirements, we designed two variant frameshift sites. The extended sequence has an additional 126 nucleotides upstream and downstream of the frameshift site. In contrast, the minimal sequence only includes 30 upstream and downstream of the frameshift site. We used cloning techniques to ligate a DNA insert coding for each frameshift site into the p2luc vector between the regions coding for two luciferase proteins - Firefly (fluc) and Renilla (rluc). In the p2luc vector, fluc is in the -1 reading frame relative to rluc. At this time, we have cloned the extended frameshift site into the p2luc vector. In order to sequence the plasmid DNA, we amplified a 700 nucleotide section of the plasmid using a method called "Slowdown PCR." This allowed the DNA polymerase to properly amplify the GC rich region within the frameshift site sequence. This method, used in cis with a decreased denaturation temperature of 90 degrees Celsius for the sequencing reaction, allowed us to sequence the 700 nucleotide PCR product. These results were compared to our intended plasmid sequence and we were able to verify successful plasmid DNA cloning. In future experiments, we will amplify this DNA, use it to synthesize RNA, and then measure the in vitro frameshift efficiency for the extended frameshift site in triplicate. These experiments will be repeated for the minimal frameshift site discussed above. Frameshift efficiencies will be compared to published values for the HTLV-II gag-pro frameshift site. Based on the results, the effect of the upstream and downstream sequence on frameshift efficiency can be determined.