Effects of the Adenovirus Type 5 Tripartite Leader Sequence with Partial and Complete Exons on mRNA Transport, Stability and Translation in Chinese Hamster Ovary Cells

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Abstract

Adenoviruses have been used as a model system for understanding gene transcription, molecular transformation, DNA replication, gene delivery and other related disciplines. These viruses have a linear double-stranded DNA genome that is transcribed into covalently closed circular RNA (cccRNA) transcripts. Subsequently, a leader sequences is required for the splicing of these transcripts. The leader region of the cccRNA is transcribed to viral pre-mRNA. Under in vitro conditions, new leader sequences were required in order to generate mature pre-mRNA. In vivo, the leader sequences were used to drive the expression of the reporter gene. The results showed that the use of the wild type leader sequence has different effects on the stability and translation of the leader sequences. This study demonstrated the importance of the leader sequence in the transcription and translation processes of the adenovirus.

Results

Figure 1: Schematic diagram of the constructs used. Each of the four constructs contains the 5’-UTR, poly(A) tail and full-length protein coding sequence (open box). In Exon-1, exon-2, and the full-length protein coding sequence, the reporter gene was inserted in the 5’-UTR, poly(A) tail and full-length protein coding sequence, respectively.

Figure 2: The effect of the leader sequence on mRNA stability in the Chinese Hamster Ovary (CHO) cell line. Each of the constructs was transfected into the CHO cells, and the mRNA stability was measured by real-time quantitative PCR. The results showed that the leader sequence had a significant effect on mRNA stability.

Figure 3: Graph showing the effect of the leader sequence on mRNA translation in the CHO cell line. Each of the constructs was transfected into the CHO cells, and the mRNA translation was measured by real-time quantitative PCR. The results showed that the leader sequence had a significant effect on mRNA translation.

Figure 4: Graph showing the effect of the leader sequence on mRNA stability and translation in the CHO cell line. Each of the constructs was transfected into the CHO cells, and the mRNA stability and translation were measured by real-time quantitative PCR. The results showed that the leader sequence had a significant effect on both mRNA stability and translation.

Conclusions

The leader sequence significantly affects the mRNA stability and translation in the CHO cell line. The results showed that the leader sequence had a significant effect on both mRNA stability and translation.

References


Methods

Four plasmid vectors (pJ200, pJ200, pJ200 and pJ200) were constructed to generate complete Ad5 mRNA. The 5’-UTR, poly(A) tail and full-length protein coding sequence were inserted into the pJ200 vector. The leader sequences were inserted into the pJ200 vector.

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