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

Adenovirous have been used as a model system for understanding gene expression, reverse transcription, DNA replication, gene delivery and other cellular processes. Among the significant steps in the adenovirus life cycle is the efficient transport of the partially processed mRNA from the nucleus to the cytoplasm. We hypothesized that specific parts of the adenovirus type 5 tripartite leader sequence (TLP) could affect mRNA transport. Therefore, we constructed a series of plasmids containing the TLP, with partial (TLP2), and complete (TLP3) TLPs. The resulting mRNA transcript was radiolabeled and nuclear runoff assays were performed. The data show that the presence of the complete TLP significantly enhances transcript nuclear export. In conclusion, the TLP is involved in mRNA transport and its components are involved in different steps, with the complete TLP playing the major role.

Introduction

The tripartite leader sequence is essential for the production of an abundant amount of pre-mRNA species required for proper RNA splicing and processing. In the absence of the leader sequence, the RNA is not exported from the nucleus and accumulates in the nucleus.

The leader sequence is responsible for the nuclear export of the pre-mRNA transcript. The TLP is composed of three segments: a 5' m7G cap, a nuclear export signal (NES), and a conserved 14-nucleotide (nt) motif (GCGCATCTCAGG). The TLP motif is essential for the proper nuclear export of the pre-mRNA transcript. The presence of the TLP on the pre-mRNA transcript is required for nuclear export.

Methods

Five plasmids (pPL2, pPL3, pPL5, and pPL6 and pPL7) were constructed with partial or complete TLP. The TLP and its variants were cloned into the pCMV-SPORT6 plasmid vector. The plasmids were transfected into 293T cells, and the resulting RNA transcripts were radiolabeled. Nuclear export assays were performed to determine the effects of the TLP on mRNA transport.

Results

Figure 1: Schematic diagrams of the constructs used. Each of the four truncated leader plasmids pPL2, pPL5, pPL6 and pPL7, as constructed a complete PreTLP, separate gene and a short (14 nt) clone in pSPORT6 plasmid. Truncated leader sequence were formed stromalin of the TLP. Snp 5 ' ends from the last gene (ene.) and the full TLP sequence were cloned to construct pSPORT6 plasmid. (A) Schematic, (B) DNA sequence.

Figure 2: 1/2 of the precursor plasmids pPL2, pPL5 (A), pPL6 (B) and pPL7 (C) contain a common preTLP, separate gene and a short (14 nt) clone in pSPORT6 plasmid. Truncated leader sequence were formed stromalin of the TLP. Snp 5 ' ends from the last gene (ene.) and the full TLP sequence were cloned to construct pSPORT6 plasmid. (A) Schematic, (B) DNA sequence.

Figure 3: Transcription efficiency of GAPDH (anti-sense strand) was determined by measuring the mRNA accumulation in the presence of TLPs. The data show that the presence of the complete TLP significantly enhances transcript nuclear export. In conclusion, the TLP is involved in mRNA transport and its components are involved in different steps, with the complete TLP playing the major role.

Figure 4: QRT-PCR mRNA reverse transcription of the different plasmids into CHO cells. Percentage accumulation rate = (Cytoplasmic precursor/mRNA accumulation rate) + (cytoplasmic precursor/mRNA accumulation rate). The data show that the presence of the complete TLP significantly enhances transcript nuclear export. In conclusion, the TLP is involved in mRNA transport and its components are involved in different steps, with the complete TLP playing the major role.

Conclusions

The results show that the presence of the complete TLP significantly enhances transcript nuclear export. In conclusion, the TLP is involved in mRNA transport and its components are involved in different steps, with the complete TLP playing the major role.

References


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