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Effects of Adenovirus Super-infection on Transgene Expression from a Gene under the MLP-TPL Promoter-Leader Sequence and the CMVie Promoter

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Abstract

Adenoviruses are commonly used as a tool for the study and understanding of gene expression, oncogenic transformation, DNA replication, gene delivery and other molecular biological phenomenon. Upon infection of permissive cells and during the late phase of the viral life cycle the adenoviral major late promoter (MLP) is the predominant promoter. The MLP, in conjunction with the tripartite leader sequence (TPL), is responsible for the abundant late viral protein production. In addition, the cytomegalovirus immediate early (CMVie) promoter drives transcription efficiency very effectively and is the most commonly used promoter for transgene expression in different cells. In this study, we investigated the gene expression activity driven by the MLP-TPL promoter-leader sequence and the CMVie promoter, under the control of the adenoviral wild type super-infection. Two plasmids were constructed and both contain the green fluorescence protein (GFP) as a reporter gene. In the first construct, GFP was driven by the MLP-TPL promoter-leader, while CMVie promoter was used in the second construct. Gene expression from both constructs was evaluated in Chinese hamster ovary (CHO) cells, in both the absence and presence of adenovirus wild type super-infection. GFP mRNA transcription levels were measured over time via RT-qPCR. Translation levels were determined by measuring GFP fluorescence intensity from the CHO cells. Upon transfection, GFP mRNA transcription efficiency and GFP intensity from the CMVie construct was significantly higher than that from the MLP-TPL. On the other hand, transfection efficiency from the MLP-TPL was enhanced transiently with the wild type adenovirus super-infection compared to stable enhancement from the CMVie construct. Moreover, GFP intensity from the CMVie construct was highly elevated with the super-infection. These results indicate that CMVie promoter is transactivated by the adenoviral proteins. This suggests that GFP transcripts from the CMVie construct were able to elude from the viral translation shut off mechanism or that such a mechanism is not as efficient in CHO cells.

Introduction

- The late phase of adenovirus infection is characterized by the production of an abundant amount of late proteins required to form and assemble the new viral capsids. Active translation in this phase is attributed to the activity of the major late promoter (MLP) and the presence of the tripartite leader sequence (TPL).
- TPL is a 5' untranslated sequence present in all of the late, but none of the early, viral mRNA. The adenovirus serotype 5 (Ad5) leader sequence is 201 bp formed by the splicing of three exons during the post-translational modifications. TPL facilitates mRNA transport and accumulation in the cytoplasm and is responsible for the selective translation of the late viral proteins in preference to the cellular proteins (10).
- Adenovirus E1B-55K and E4-orf6 play the main role in active transport of TPL-containing mRNA from the nucleus to the cytoplasm (1, 8). The viral transcription sites in the nucleus contain a complex of E1B-55K and E4-orf6 (6). Evidence suggests that viral mRNA interacts with this complex through the ability of E1B-55K to bind RNA (7), and facilitate its transport to the cytoplasm using the E4-orf6 proteins nuclear localization and transport signals (2). Cellular mRNA transport is blocked by the same complex (5).
- Translation of any TPL-attached mRNA is eIF-4F-independent (4). The relaxed secondary structure of TPL facilitates its function in translation initiation even when eIF-4F is inhibited (3).
- The CMVie promoter has been made the most commonly used promoter in transgene expression in different cells due to its very efficient transcription efficiency (9).
- Since these elements are used in different gene therapy constructs, it is important to understand the effect of the common natural adenoviral infection on gene expression under the control of these elements. Therefore, we constructed two plasmids with green fluorescence protein (GFP) gene driven by either the MLP-TPL promoter-leader or the CMVie promoter. Wild type adenovirus dl309 was used to super-infect these plasmids in Chinese hamster ovary (CHO) cells.

Methods

- Two plasmids (pMTGA and pCG) were constructed Ad5 MLP-TPL promoter-leader and CMVie promoter, respectively (Figure 1). Plasmids were prepared by CsCl gradients.
- Equal amounts from each plasmid were transfected into CHO cells using the calcium phosphate method. Transfection was done in two identical groups, each as triplicate wells in 6-well plates, and the medium was replaced 6 h post-transfection.
- 12 h post-transfection, one of the groups were super-infected with the wild type dl309 at a multiplicity of infection (MOI) of 1 plaque forming unit (PFU)/cell.
- Total RNA and DNA were isolated from the transfected and transfected/super-infected cells, using Norgen's RNA/DNA/Protein Purification Kit (Norgen Biotek. Corp., Thorold, ON, Canada). Samples were collected after 0, 12, 36 84 hours, 7.5, 11.5 and 15.5 days post-transfection.
- All isolated RNAs were treated with Ambion`s turbo DNase (Ambion, Austin, TX, USA) to digest any residual DNA background and were then cleaned using Norgen`s RNA Clean-Up and Concentration Kit (Norgen Biotek. Corp., Thorold, ON, Canada). Specific PCR for the GFP fragment was used to check the success of the digestion step.
- qPCR and qRT-PCR were performed on the isolated DNA and RNA samples, using 1x SYBR GREEN master mix (Bio-Rad, Hercules, CA, USA), and specific primers for GFP fragment (Forward: 5' ATCCTGATCGAGCTGAATGG 3' and Reverse: 5' TGCCATCCTCGATGTTGTG 3'). Reactions were performed using Bio-Rad iCycler thermal cycler.
- The fluorescence intensity of green fluorescence protein (GFP) was quantified directly from mammalian cells by measuring the relative fluorescence units (RFU) using the BioTek Synergy HT Multi-Mode Microplate Reader. Transfected cells were washed twice with PBS, lifted from the plate and counted. Fifty thousand cells per well were then transferred to a black rounded-bottom 96-well plate (Costar) in a total volume of 200 µL of PBS. The RFU was then measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm, using non-transfected cells as a blank.

Results

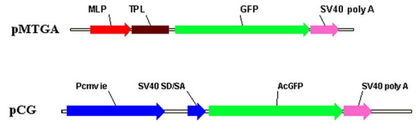

Figure 1: Schematic diagrams of the constructs. The two plasmids contain a common gene (GFP) and poly A signal (SV40 poly A). Different regulatory elements are used to drive the expression, either MLP-TPL (pMTGA) or CMVie (pCG).

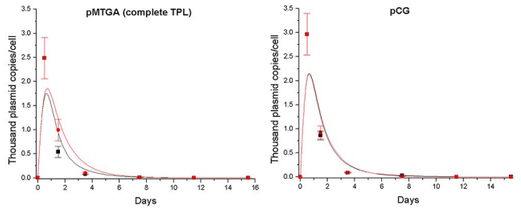

Figure 2: Copy numbers of the two plasmids over 15.5 days post-transfection into CHO cells, with transfection and super-infection conditions. Copy numbers were obtained by qPCR using a standard curve of known plasmid DNA concentration. qPCR was performed on equal amounts of DNA isolated from collected samples. Plasmids names are shown on the figure with transfection (—) and super-infection (—) conditions.

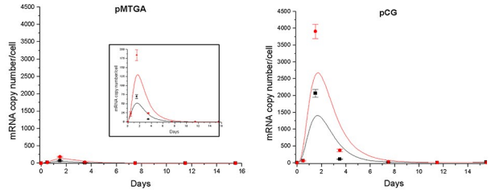

Figure 3: GFP mRNA transcripts from the two plasmids over 15.5 days post-transfection into CHO cells, with transfection and super-infection conditions. Copy numbers were obtained by qPCR using a standard curve of known plasmid DNA concentration. qPCR was performed on equal volumes of RT product from equal amount of RNA isolated from collected samples. Plasmids names are shown on the figure with transfection (—) and super-infection (—) conditions.

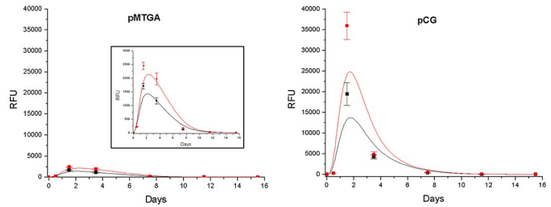

Figure 4: GFP mRNA transport rate over 24 hours post-transfection of the different plasmids into CHO cells. mRNA copy numbers were measured by qRT-PCR, using a standard curve of known concentrations. Transport rate was calculated as the percentage of cytoplasmic mRNA over the sum of cytoplasmic and nuclear mRNA.

Conclusions

- Wild type adenovirus super-infection does not affect plasmid stability in the two tested constructs.
- CMVie is more active than the MLP-TPL in absence of the adenoviral super-infection. This activity is about 20 folds higher on the mRNA transcription level and about 11 levels on the translation level, within the increment peak. The presence of the adenoviral TPL plays a role in this fold difference reduction.
- Adenoviral super-infection significantly (at P<0.05) enhances mRNA levels as well as GFP translation from both constructs, when compared to their non-infected conditions (about 2.6 & 1.9 folds on the transcription level and 1.6 & 1.9 folds on the translation level from pMTGA & pCG, respectively).
- Under the super-infection, CMVie continued to show higher activity than MLP-TPL, with about 21 & 15 fold increases on the transcription and translation levels, respectively.
- It seems that adenoviral infection enhances gene expression driven by the CMVie promoter. This enhancement could result from the direct interaction between the CMVie and adenoviral proteins and elements or indirectly through viral effects on the cellular environment

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