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ORIGINAL ARTICLE

Effect of adenovirus infection on transgene expression under the adenoviral MLP/TPL and the CMVie promoter/enhancer in CHO cells

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Abstract The adenovirus major late promoter (MLP) and its translational regulator – the tripartite leader (TPL) sequence – can actively drive efficient gene expression during adenoviral infection. However, both elements have not been widely tested in transgene expression outside of the adenovirus genome context. In this study, we tested whether the combination of MLP and TPL would enhance transgene expression beyond that of the most widely used promoter in transgene expression in mammalian cells, the cytomegalovirus immediate early (CMVie) promoter/enhancer. The activity of these two regulatory elements was compared in Chinese hamster ovary (CHO) cells. Although transient expression was significantly higher under the control of the CMVie promoter/enhance compared to the MLP/TPL, this difference was greater at the level of transcription (30 folds) than translation (11 folds). Even with adenovirus infection to provide additional elements (*in trans*), CMVie promoter/enhancer exhibited significantly higher activity relative to MLP/TPL. Interestingly, the CMVie promoter/enhancer was 1.9 folds more active in adenovirus-infected cells than in non-infected cells. Our study shows that the MLP-TPL drives lower transgene expression than the CMVie promoter/enhancer particularly at the transcription level. The data also highlight the utility of the TPL sequence at the translation level and/or possible overwhelming of the cellular translational machinery by the high transcription activity of the CMVie promoter/enhancer. In addition, here we present data that show stimulation of the CMVie promoter/enhancer by adenovirus infection, which may prove interesting in future work to test the combination of CMVie/TPL sequence, and additional adenovirus elements, for transgene expression.

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1. Introduction

Active gene expression is an essential requirement in different applications including DNA vaccines, gene therapy and commercial protein production. The choice of promoters as well as other regulatory sequences that act in combination to derive active gene expression is a major optimization target to facilitate the outcomes of the transgene construct. The adenovirus major late promoter (MLP) and the cytomegalovirus immediate early (CMVie) promoter/enhancer are two of the most commonly used promoters in transgene expression [25,29]. The late phase of adenovirus infection starts when the L4-22K and L4-33K proteins are expressed at low levels to fully activate the MLP [27,46]. Once activated, the MLP will derive the expression of an abundant amount of late proteins required to form and assemble new viral capsids. Active translation in this phase is attributed to the activity of the MLP and the presence of the tripartite leader sequence (TPL). The expression of all the late viral proteins is driven by the MLP which has its full activity during the late phase of the viral infection and is transactivated by the adenoviral E1A proteins [30,51].

TPL is a 5′ untranslated sequence present in all of the late, but none of the early, viral mRNA. TPL facilitates mRNA transport and accumulation in the cytoplasm and is responsible for the selective translation of the late viral proteins in preference of the cellular proteins [49]. Translation of any TPL-attached mRNA is eIF-4F-independent [10]. The relaxed secondary structure of TPL facilitates its function in translation initiation even when eIF-4F is inhibited [11].

The CMVie promoter/enhancer is the most commonly used promoter for transgene expression in mammalian cells because it achieves high levels of transcription [1,5,6,14,19,33,34,38,40,44,45]. In the context of cytomegalovirus replication, the CMVie controls the expression of the IE1, which is a transactivator essential for viral latency and replication and belongs to the immediate early family of genes that are expressed first after infection [39,41]. In this study, we engineered two constructs that contained either the MLP/TPL sequence or the CMVie promoter/enhancer for the expression of a GFP reporter gene. We then compared gene expression levels between the two constructs in non-infected and adenovirus-infected Chinese hamster ovary (CHO) cells.

2. Materials and methods

2.1. Plasmid constructs

Two plasmids were used in this study denoted by pCG and pMTGA. pCG was constructed from pCMV-GFP plasmid that was used in a previous study in our laboratory [15]. We constructed pCG plasmid by the removal of the SV40 SD/SA fragment using SacI and AgeI and closing the plasmid by cloning of the annealing product of the following two fragments 5′cgtttagtgaaccgtcagatcgcctga3′ and 5′tcgagcaggcgatctg acggttcactaaac3′. The resulting plasmid contained CMVie promoter/enhancer, green fluorescence protein (GFP) and SV40 poly A signal. On the other hand, pMTGA was constructed by the cloning of two fragments into pUC19. The first fragment contained GFP-SV40 poly A and was obtained by PCR on pCG plasmid using the following two primers: 5′atg gtgagcaagggcgc3′ and 5′ttgttgttaacttgtttattgcagcttataatgg3′. The obtained fragment was cloned into the HincII site of pUC19. Next, a fragment that contains the major late promoter (MLP) and tripartite leader sequence (TPL) was obtained by gene construction and cloned into NheI and AgeI sites, upstream from the GFP-SV40 poly A. Both plasmids were prepared by cesium chloride gradient. The schematic diagrams of the two plasmids are shown in Fig. 1.

2.2. Cell lines and maintenance

The used Chinese hamster ovary (CHO) cells were the subclone K1 (ATCC CCL-61) derived from the parental cell line initiated by Puck and coworkers [35]. Cells were maintained as a monolayer in Petri cell culture dishes and cultured in advanced Dulbecco's Modified Eagle Medium (Advanced D-MEM: Invitrogen Corp., Gibco), containing 5% (v/v) fetal bovine serum (FBS, PAA Laboratories Inc.), 1% (v/v) penicillin/streptomycin (Invitrogen Corp., Gibco) and 1% (v/v) glutamine (Invitrogen Corp., Gibco). Growing cells were incubated in a water-jacketed incubator (Fisher Scientific,

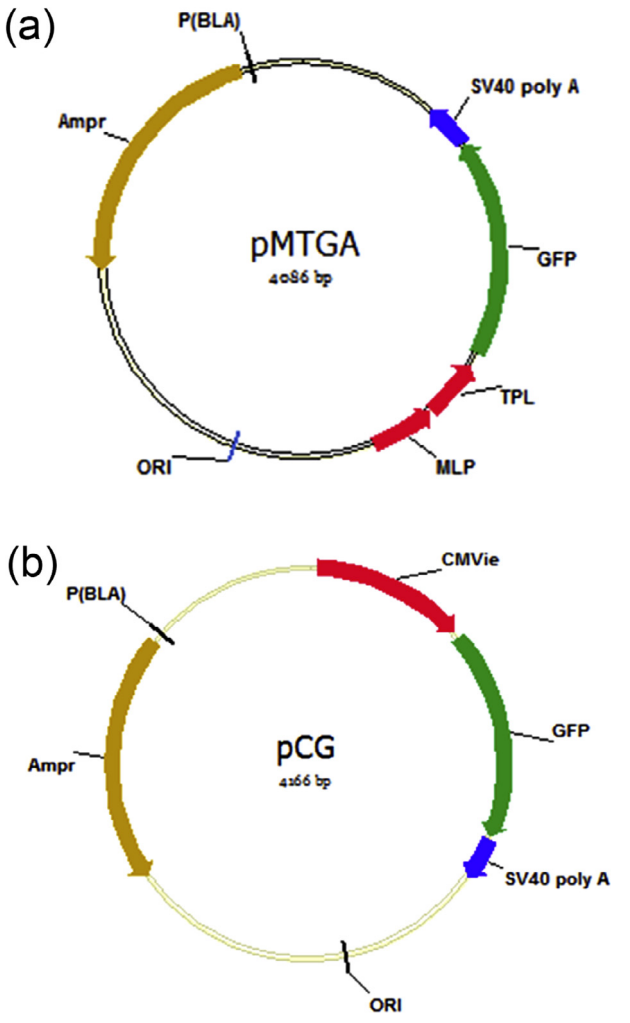

Figure 1 Schematic diagrams of pMTGA (a) and pCG (b). Both plasmids contain a common reporter gene (GFP) and poly A signal (SV40 poly A). Different regulatory elements are used to drive the expression, either the MLP-TPL (pMTGA) or the CMVie promoter/enhancer (pCG).