Available online at www.sciencedirect.com
ScienceDirect
Protein Expression and Purification 56 (2007) 269–278
Protein Expression & Purification
www.elsevier.com/locate/yprep

High level protein expression in mammalian cells using a safe viral vector: Modified vaccinia virus Ankara

Matthias Hebben [a,1], Jan Brants [a], Catherine Birck [a], Jean-Pierre Samama [a], Bohdan Wasylyk [a], Danièle Spehner [a], Karine Pradeau [a], Arban Domi [b], Bernard Moss [b], Patrick Schultz [a], Robert Drillien [a,*]

[a] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM U596/CNRS-UMR7104, 1 rue Laurent Fries, 67404 Illkirch, Université Louis Pasteur, Strasbourg, France*
[b] *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA*

Received 25 May 2007, and in revised form 27 July 2007
Available online 24 August 2007

Abstract

Vaccinia virus vectors are attractive tools to direct high level protein synthesis in mammalian cells. In one of the most efficient strategies developed so far, the gene to be expressed is positioned downstream of a bacteriophage T7 promoter within the vaccinia genome and transcribed by the T7 RNA polymerase, also encoded by the vaccinia virus genome. Tight regulation of transcription and efficient translation are ensured by control elements of the *Escherichia coli* lactose operon and the encephalomyocarditis virus leader sequence, respectively. We have integrated such a stringently controlled expression system, previously used successfully in a standard vaccinia virus backbone, into the modified vaccinia virus Ankara strain (MVA). In this manner, proteins of interest can be produced in mammalian cells under standard laboratory conditions because of the inherent safety of the MVA strain. Using this system for expression of β-galactosidase, about 15 mg protein could be produced from 10^8 BHK21 cells over a 24-h period, a value 4-fold higher than the amount produced from an identical expression system based on a standard vaccinia virus strain. In another application, we employed the MVA vector to produce human tubulin tyrosine ligase and demonstrate that this protein becomes a major cellular protein upon induction conditions and displays its characteristic enzymatic activity. The MVA vector should prove useful for many other applications in which mammalian cells are required for protein production.

Keywords: Vaccinia virus; Modified vaccinia virus Ankara (MVA); Bacteriophage T7; Protein expression; Tubulin tyrosine ligase

A variety of expression systems have been developed to overproduce proteins in mammalian cells. Viral vectors are particularly powerful tools because they have inbuilt mechanisms to subvert the cellular machinery in their favour. Vaccinia virus (VACV)[2], the prototype of the Poxviridae family, has been used for more than 20 years as an expression vector [1–3] and it displays a number of advantages for such applications. Like all Poxviruses, VACV replicates entirely in the cytoplasm of the host cell where transcription and replication of the viral DNA genome are carried out by virus encoded machinery. This property accounts in part for the ability of VACV to infect a wide range of cell types from different species. Furthermore, the size of the genome packaged into viral particles is not precisely controlled so that as much as 25 kbp can be accommodated beyond the approximately 200 kbp normally encapsidated [4]. In numerous strategies for expression of foreign genes using VACV, the gene of interest has been positioned downstream of VACV early or late promoters.

* Corresponding author. Fax: +33 3 88 65 32 01.
E-mail address: drillien@igbmc.u-strasbg.fr (R. Drillien).
[1] Present address: Intervet Pharma R&D, Rue Olivier de Serres, BP 67131, 49071 Beaucouzé Cedex, France.
[2] *Abbreviations used:* VACV, vaccinia virus; IPTG, isopropyl β-D-thiogalactoside; WR, western reserve; FCS, fetal calf serum; hTTL, human tubulin tyrosine ligase; MVA, modified vaccinia virus ankara strain; MEM, minimum essential medium; CEF, chicken embryo fibroblasts; PFU, plaque forming units; GFP, green fluorescent protein; βgal, β-galactosidase; BHK21, baby hamster kidney; MUG, 4-méthylumbelliferyl β-D-galactoside; TK, thymidine kinase; TBP, tata binding protein.

1046-5928/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2007.08.003

However, the highest expression levels have been achieved when the gene encoding the RNA polymerase from the bacteriophage T7 was integrated into the VACV genome and the foreign gene of interest was under the control of a bacteriophage T7 promoter [5,6]. To isolate viable virus encoding within the same genome both the T7 RNA polymerase and a foreign gene under the control of a T7 promoter it was necessary to tightly regulate expression of the T7 RNA polymerase by regulatory elements of the lactose operon, namely a lac operator positioned upstream of the RNA polymerase gene and a lac repressor under the control of a VACV promoter [7]. The level of expression of the foreign gene could then be controlled by the concentration of the lactose analogue, isopropyl β-D-thiogalactoside (IPTG), added to the culture medium. Further improvements of protein expression were accomplished by adding an encephalomyocarditis virus leader sequence upstream of the foreign gene [8–10] and more stringent control of expression was obtained by positioning an additional lac operator upstream of the foreign gene [8]. In addition, transcription termination was improved by adding a strong rho-independent ribosomal transcription terminator downstream of the foreign gene [8]. Under these conditions, Ward et al. [8] showed that as much as 2 mg protein could be produced from 10^8 infected cells over a 24-h period. The tightly regulated VACV-T7 expression system developed so far has been built into the Western reserve (WR) strain of VACV which displays the broad host range typical of VACV and is potentially pathogenic for individuals with immune system or skin disorders. In fact, most regulatory authorities require that the WR strain be handled under biosafety level 2 containment conditions. To avoid such hazards and yet be able to produce large amounts of recombinant protein, the modified vaccinia virus Ankara strain (MVA), which is unable to significantly multiply in human cells [11,12], is safe for highly immunocompromised animals [13,14] and can be handled under biosafety level 1 containment conditions, has been engineered to encode bacteriophage T7 RNA polymerase [15,16]. However, the MVA-T7 expression system has not been optimized by addition of lactose operon elements nor for efficient translation by use of the encephalomyocarditis virus leader. Here, we report optimization of the MVA-T7 expression system by introducing the same regulatory elements developed for high level expression using the VACV-WR strain [8]. During these studies we have found that the optimized MVA-T7 expression system, in addition to its intrinsic safety, displays the added benefit of inducing 4-fold higher levels of foreign protein synthesis than the same system in a VACV-WR background. To further demonstrate the usefulness of the MVA-T7 vector system, we have employed it to express human tubulin tyrosine ligase (hTTL), a protein not previously expressed to high levels in *Escherichia coli* or mammalian cells.

Materials and methods

Viruses and cells

The Baby Hamster Kidney (BHK21) cell line was cultivated in the Glasgow modification of Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS) and 1.5 g/ml bacto-tryptose phosphate. Human HeLa cells were grown in MEM supplemented with 10% FCS. Primary chicken embryo fibroblasts (CEF) were prepared from 11-day-old embryos and grown in Eagle's Basal Medium supplemented with 10% FCS and 1.5 g/ml bacto-tryptose phosphate. The MVA-N33 strain was generously provided by J.-M. Balloul (Transgène SA, Strasbourg, France). MVA-N33 was derived from a lyophilized vaccine preparation (MVA II/85 provided by Anton Mayr) by two successive plaque purifications on CEF at Transgène and was shown to display the characteristic features of previously published MVA isolates ([17] and data not shown). The VACV-WR strain was obtained from the American Type Culture Collection. All viruses were propagated on BHK21 cells. For titrations, the culture medium was removed from 35 mm tissue culture plates containing BHK21 cell monolayers. Serial dilutions of virus samples were then deposited on the cells for 1 h at room temperature. Fresh culture medium was then added to the dishes which were incubated for 2 days at 37 °C. The medium was then removed, the cells stained with 4% neutral red and plaques counted under a microscope. Virus titers were expressed in plaque forming units (PFU) per ml.

Plasmids

The pgT7lacOI-TK.R-L plasmid (unpublished data, A.D. and B.M.), is essentially identical to the plasmid pgT7lacOI encoding both the bacteriophage T7 RNA polymerase and the Lac I repressor that was used previously to insert these genes into the WR strain [7]. Foreign genes interrupt the viral thymidine kinase locus in the pgT7lacOI-TK.R-L plasmid, thus enabling selection of recombinant vaccinia virus for a thymidine kinase negative phenotype. However, this property could not be used easily for selection of recombinant MVA viruses for lack of a thymidine kinase deficient cell line that is sensitive to MVA infection. Therefore, we added a gene encoding the green fluorescent protein (GFP) as a selective marker to pgT7lacOI-TK.R-L by cutting the latter at its unique SmaI site and inserting a NaeI fragment containing the GFP gene (Clontech) downstream of a VACV synthetic early promoter [18,19]. This procedure generated a plasmid named pEM24. The pVote2 plasmid has been developed for transfer of a tightly controlled T7 expression cassette to the hemagglutinin locus of the VACV genome [8]. A lacZ cassette obtained by cutting pMC1871 (GenBank Number: L08936.1) with BamHI was inserted in frame into the unique BamHI site of pVote2 and the new plasmid was designated pEM28. The gene encoding human tubulin