CHEM. RES. CHINESE UNIVERSITIES
2008, 24(1), 75—79
Article ID 1005-9040(2008)-01-075-05

Available online at www.sciencedirect.com
ScienceDirect

Translational Enhancer of *Tobacco mosaic virus* Enhancing Expression of Hepatitis B Surface Antigen in Transgenic Panax ginseng C. A. Meyer Callus

GAO Zheng-lun[1], SHENG Jun[2*], HAO Shu-mei[3], LIU Dan[2], LIU Xiao-yu[2], JI Hai-bin[2], LI Juan[2] and ZHANG Xian-ping[4]

1. College of Life Science, Jilin University, Changchun 130012, P. R. China;
2. Changchun Institute of Biological Product, Changchun 130062, P. R. China;
3. Changchun University of Science and Technology, Changchun 130022, P. R. China;
4. Jilin Institute for Food and Drug Control, Changchun 130062, P. R. China

Abstract The 5′-nontranslated leader(omega sequence) of *Tobacco mosaic virus*(TMV) was used as a translational enhancer sequence in the expression of the hepatitis B surface antigen(HBsAg) gene in transgenic ginseng callus cultures. The *adr* subtype HBsAg gene was placed under the control of the *Cauliflower mosaic virus*(CaMV) 35S promoter linking to the TMV leader sequence. The antisense omega sequence was used in a control construct. The resulting constructs cloned in the binary vector pBI121 were used to transform the ginseng *callus tissue via* the *Agrobacterium*-mediated procedure. The integration and expression of the HBsAg gene were evaluated by PCR and western blot, respectively. Enzyme-linked immunoassays(ELISA) using a monoclonal antibody directed against human serum-derived HBsAg revealed a three to four-fold enhanced expression of HBsAg in ginseng cells conferred by the TMV omega element.

Keywords Translational enhancer; Omega sequence; Hepatitis B surface antigen; Ginseng callus

1 Introduction

Hepatitis B virus infection is one of the most common viral infections affecting humans, and is particularly widespread in China[1]. The intramuscular injection of CHO-derived HBsAg or yeast-derived HBsAg into healthy individuals generates effective immunization and protection from viral infection; however, there is still an urgent need for a more affordable and reliable vaccine[2]. Over the past decade, the expression of subunit vaccine antigens in plants has emerged as a convenient, safe, and potentially economical platform technology, with the potential to provide a novel biotechnological solution to vaccine production and delivery[3]. As an alternative to the administration of vaccines by needle and syringe, oral vaccines offer significant advantages: including simplicity of use, increase in compliance, enhanced immune responses at mucosal sites, and stimulation of humoral immunity[4]. Hepatitis B surface antigen has been successfully expressed in plants[5] and delivered orally in both animal[6] and human[7].

However, one of the major limitations of the expression of recombinant antigens in transgenic plants remains the achievement of antigen-yields high enough to confer total protection in humans. This has led us to develop a system for the high-level production of rHBsAg in ginseng cell cultures, which is vital for their use as potential vaccines.

Panax ginseng C. A. Meyer, a perennial herb, commonly known as "ginseng" has been widely used as a tonic and herbal medicine particularly in Korea and China since ancient times. It also contains various saponins and sapogenins[8]. It is effective against gastroenteric disorders, diabetes, and weak circulation. It has been used as an adjuvant to prevent various forms of hepatitis[9]. Owing to the relatively long growing cycle of ginseng, four to seven years in the field, we have produced ginseng cells through plant tissue cell cultures.

The 5′-nontranslated leader of TMV RNA contains a highly organized 68-base region, which has been shown to act as an enhancer of both eukaryotic and prokaryotic translation *in vivo*[10] and *in vitro*[11]. This element, known as the omega leader, is thought

* Corresponding author. E-mail: gzlun418@yahoo.com.cn
Received April 9, 2007; accepted June 29, 2007.
Supported by the Science and Technology Development Planning Foundation of Jilin Province, China(No. 20030405).

Copyright © 2008, Jilin University. Published by Elsevier Limited. All rights reserved.

to influence the effectiveness with which bound 40S ribosomal subunits migrate and recognize the translational start site. Moreover, the degree to which the omega leader enhances translation is highly species-dependent. The enhancement is higher in dicotyledonous plant cells than in monotyledonous cells[12].

Herein is a transgenic system based on the efficient omega leader element for the high-level production of HBsAg in ginseng callus(dicots). This represents an important technological development for the subsequent use of ginseng in vaccine delivery.

2 Material and Methods

2.1 Establishment of Ginseng Cell Cultures

Ginseng cell line was obtained from Institute of Plant Physiology & Ecology, Shanghai, China. The callus was maintained on 67-V semi-solid medium composed of MS salt[13], 87.7 mmol/L sucrose, 1.0 g/L lactalbumin hydrolysate, without plant growth regulator. The pH of the medium was adjusted to 5.8, and the 7—10 days post-subculture cells on semi-solid medium were used for transformation.

2.2 Synthesis of 5′-Nontranslated Leader

The omega(68 bp) leader from the U1 strain of TMV was synthesized by Sangon, China. We changed CAU in nature nucleotides[Fig.1(A)] to CAA[Fig.1(B)] lest the open reading frame should exist in reverse nucleotide sequence[Fig.1(C)].

(A) 5′-UAUUUUUACA ACAAUUAC *CAACAACCAACAACAAACAACAAACAA* CAU UCAAUUAC*UAUUU*ACAAUUACA-3′

(B) 5′-G(*BamHI/BglII* fusion)GATCTTATTTTTACA ACAATTAC*CAACAACAACAACAAACAACAAACAA*CAATACAATTACTATTTACAATTACAGGATCC(*Bam*HI)-3′

(C) 5′-GGATCC(*Bam*HI) TGTAATTGTAAATAGTAATTGTATTGTTGTTTGTTGTTTGTTGTTGTTGGTAATTGTTGTAAAAATA(*Bam*HI/*Bgl*II fusion)GATCC-3′

Fig.1 Nature(A), forward(B) and reverse(C) omega nucleotide sequence of 5′-nontranslated leader

Eight-base direct repeats are underlined, and (CAA)$_n$ regions are shown in italics. Changed bases are indicated with a box.

2.3 Construction of Plasmid for Plant Transformation

The HBsAg fragment from the CHO cell line that produced rHBsAg(*adr* subtype) for use in vaccines was subcloned into pBluescript SK+(*Pst*I/*Sac*I). Thus, the HBsAg "s" gene was released by digestion with *Eco*RV/*Sst*I. pBI121(obtained from Clontech) was digested with *Sma*I/*Sst*I, and then the vector was isolated to yield GUS-less pBI121. The HBsAg gene was ligated into the vector to form pBI(Fig.2).

The CaMV 35S promoter linked to the forward and the reverse omega leader, respectively, which acted as a translation enhancer[14], was constructed as shown in Fig.2. The plasmids contained the left and right border regions, which denoted the limits of the DNA *via Agrobacterium tumefaciens*-mediated transformant, as well as the neomycin phosphotransferase gene, which allowed selection with kanamycin.

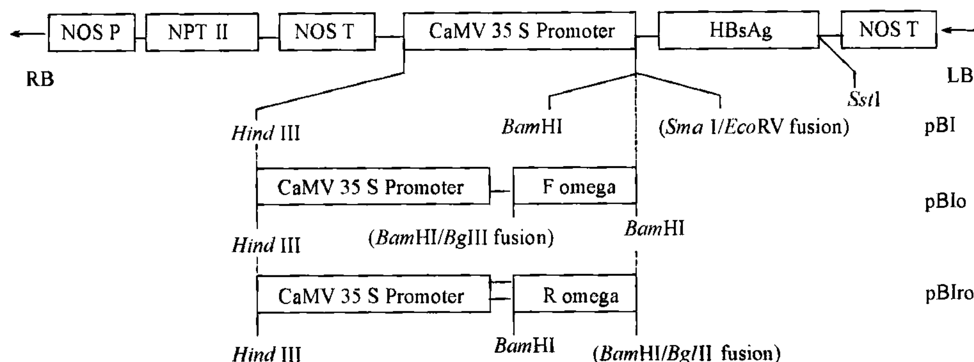

Fig.2 Structure of plasmids pBI, pBIo, and pBIro

Constructs carry the left and right borders(LB, RB) of the transferred DNA, which demarcates the fragments that are incorporated into the plant genomic DNA *via Agrobacterium*-mediated transformation. 5′-Nontranslated leaders lie downstream of the CaMV 35S promoter and are followed by the HBsAg coding region. In pBIo, the 35S promoter is linked to the TMV omega 5′-nontranslated leader. In pBIro, the 35S promoter is linked to the reverse omega. Abbreviations: F: forward; R: reverse. NOS P: nopaline synthase promoter; NPTII, neomycin phosphotransferase II; NOS T: nopaline synthase terminator; LB: left border; RB: right border; HBsAg: hepatitis B surface antigen.

Download English Version:

https://daneshyari.com/en/article/1253218

Download Persian Version:

https://daneshyari.com/article/1253218

Daneshyari.com