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Pleiotrophic functions of Epstein-Barr virus nuclear antigen-1 (EBNA-1) and oriP differentially contribute to the efficacy of transfection/expression of exogenous gene in mammalian cells

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Abstract

The EBNA1 gene and oriP sequence, originally derived from the EBV genome, provide plasmid vectors with artificial chromosome (AC)-like characteristics, including cytoplasm-to-nuclear transport, nuclear retention, replication and segregation of the DNA, while transcriptional up-regulation has been suggested as another activity of the EBNA1/oriP. Transfection as well as expression rates of various nonviral delivery vehicles are highly improved by inserting these genetic elements into plasmid DNA constructs. Here we differentially analyzed the contribution of each function of the EBNA1/oriP to the efficacy of electroporation-mediated genetic delivery and expression in mammalian cells. It was found that the EBNA1/oriP-mediated acceleration of genetic delivery and expression was predominantly due to the promotion of cytoplasm-to-nuclear recruitment as well as enhancement of transcription, while the episomal replication of the EBV-AC was not essentially involved.

Keywords: EBNA1; Transfection; Artificial chromosome

1. Introduction

To analyze and manipulate the mammalian cell functions, efficient and high-throughput technologies to transfer genetic information into cells and tissues are required. However, the critical impediment is that the efficacies of delivery and transgene expression have been limited, as far as conventional nonviral delivery systems are used.

The EBNA1 gene and oriP are important genetic elements of the Epstein-Barr virus (EBV). EBNA1 is a nuclear phosphoprotein with sequence-specific DNA binding activity, while its consensus binding motifs are repetitively present in the dyad symmetry (DS) and the family of repeats (FR) elements in the oriP (reviewed in Mazda, 2000; Tsurumi et al., 2005). EBNA1 is the only viral protein necessary and sufficient to induce replication and maintenance of the EBV genome in the cells latently infected with the virus. Insertion of EBNA1 gene and oriP sequence into recombinant plasmid DNA provides the constructs with artificial chromosome (AC)-like characteristics (reviewed in Mazda, 2002; Mazda and Kishida, 2005). We previously showed that the transfection/expression efficiency of nonviral delivery systems is also highly improved by using EBV-based AC, when they were transfected into cultured cells by means of the cationic polymer, cationic liposome or electroporation (reviewed in Mazda, 2000; Mazda and Kishida, 2005). The EBV-AC also enables quite efficient genetic transduction *in vivo* into a variety of organs and tumors in animals, using synthetic compounds, cationic emulsion, electroporation, gene gun, sonoporation and naked DNA procedures (reviewed in Mazda, 2002; Mazda and Kishida, 2005).

The mechanisms underlying the efficient transfection/expression have not been fully understood, however, because the EBNA1 exerts a variety of functions through sequence-specific binding to oriP. Although EBNA1 was originally shown

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to support DNA replication in latently infected human cells (Lupton and Levine, 1985; Rawlins et al., 1985; Reisman et al., 1985; Yates et al., 1985), it was also demonstrated that the EBNA1 promotes cytoplasm-to-nuclear entry of the oriP-bearing plasmid (Ambinder et al., 1991; Fischer et al., 1997), retention of the plasmid in the nucleus (Krysan et al., 1989; Jankelevich et al., 1992; Middleton and Sugden, 1994), and transcription (Reisman and Sugden, 1986; Sugden and Warren, 1989; Wysokenski and Yates, 1989; Gahn and Sugden, 1995; Puglielli et al., 1996). These functions may partly contribute to the efficient transfection and expression of the EBV-based plasmid vectors, but the contribution of each function remains to be clarified.

In the present study we investigated the mechanisms underlying the high-level gene transfer and expression achieved by the EBNA1/oriP. The results may provide fundamental rationale of the mechanisms of the EBV systems in genomic manipulation of cells and tissues. Moreover, the results may also offer important information to devise and improve nonviral gene manipulation systems.

2. Material and method

2.1. Plasmids

The pGVP (Fig. 1, upper left) contains the Luc gene downstream of the SV40 minimal promoter. DNA fragments corresponding to oriP (AccI-BamHI 2.5 kb), FR (HaeIII-HaeIII 0.86 kb) and DS (EcoRV-ApaI 0.25 kb) were inserted into the pGVP to generate pGVP-oriP, pGVP-FR and pGVP-DS, respectively (Fig. 1, upper left). Other plasmids were previously described (Fig. 1) (Tomiyasu et al., 1998; Cui et al., 2001; Kishida et al., 2001). Plasmids were purified using Qiagen Max-iPrep Endo-free kits (Qiagen, Hilden, Germany).

2.2. Cells

HeLa (a human cervical carcinoma cell line), NIH3T3 (a murine fibroblast cell line), CT-26 (a murine colon carcinoma cell line), K562 (a human erythroleukemia cell line) and B16 (a murine melanoma cell line) were maintained under standard conditions. Transfectants constitutively expressing EBNA1 (HLE, CT-26E, 3T3E and B16E) were established from HeLa, CT-26, NIH3T3 and B16 cells, respectively, as previously described (Mazda et al., 1994; Mazda et al., 1997). Luc gene stable transfectants were established by co-transfecting HeLa, CT-26 and NIH3T3 cells with linearized pGVP-oriP and pHyg (a plasmid encoding Hygromycin resistant gene) followed by Hygromycin selection (HeLa/oriP-Luc, CT-16/oriP-Luc and 3T3/oriP-Luc cells, respectively). For cell-cycle synchronization, cells were cultured in FBS-free medium for 1 day. After washing, the culture supernatant was replaced by fresh medium supplemented with 10% FBS and 5 mg/ml of aphidicholine, followed by culturing for another 1 day.

2.3. Transfection and reporter assays

Electroporation was performed as described (Mazda et al., 1997). Luc and β-gal assays were performed as described elsewhere (Kishida et al., 2001; Nakanishi et al., 2003).

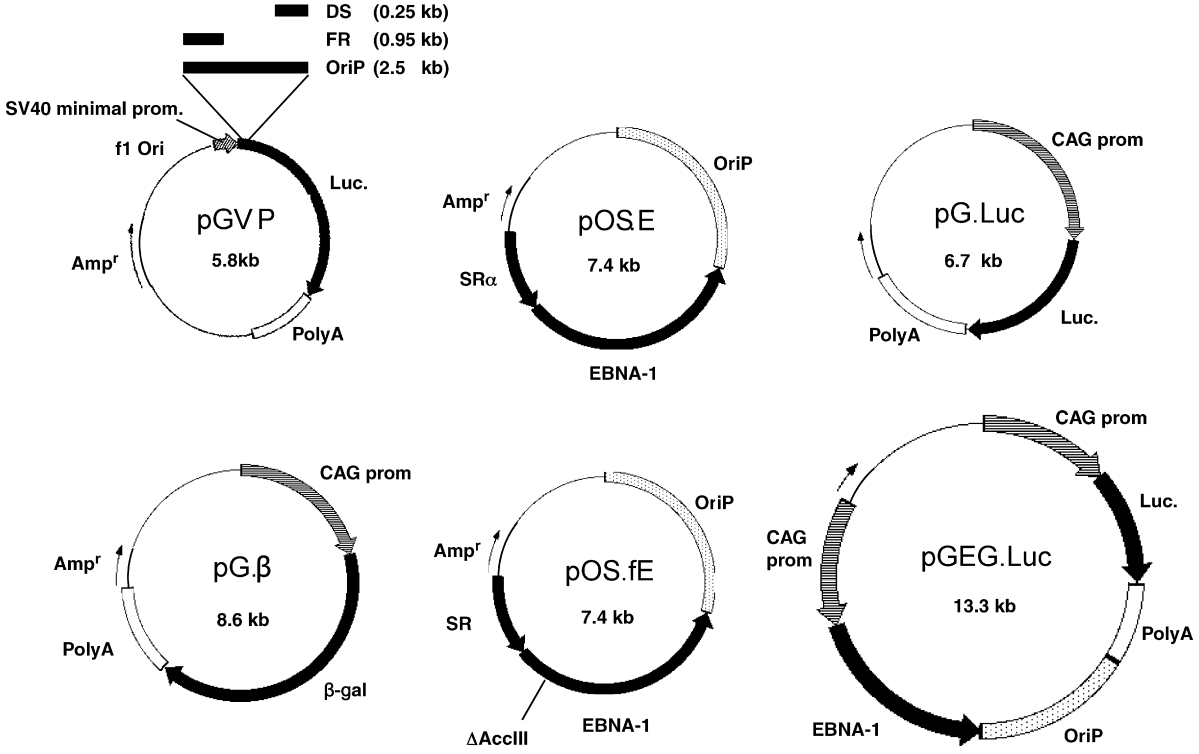

Fig. 1. Schematic diagrams of the plasmid vectors. Constructions of pGVP with/without oriP-derived fragments (upper left), pG.β (lower left), pOS.E (upper right) and pOS.fE (lower right) are represented. Prom: promoter; polyA: SV40 polyA additional signal.