Over-Expression of Protein Kinase Bα Enhances Recombinant Protein Expression in Transient Systems

ELHAM ETTEHADIEH, SHARON WONG-MADDEN, TERI ALDRICH, KELLY LANE and ARVIA E. MORRIS
Immunex Corporation, Department of Cell Sciences 51 University St. Seattle, WA 98101 U.S.A.

Abstract. As more genes are being identified through genomic techniques, the need to rapidly express recombinant proteins for functional studies has become increasingly acute. Transient expression of recombinant protein using COS-1, CV-1 and 293 cells is widely used to address this need. To improve the robustness of host cells for transient expression, the effect of over-expression of Protein Kinase Bα has been explored. In this report we demonstrate that over-expression of PKBα can improve transient recombinant protein expression 40% to >200% depending on the protein being expressed and the cell line used.

1. Introduction

COS, CV-1 and 293 cells are widely used in transient transfection systems for rapid expression of recombinant proteins in mammalian cells. Using expression plasmids capable of replication in the host cells can optimize the level of recombinant protein expression. An inherent limitation of these systems is that the viability of the host cells is often difficult to maintain, and low viability may result in loss of recombinant protein titer over time. Recent studies have shown that Protein Kinase Bα (PKBα) plays a central role in mediating cellular responses to a variety of stimuli including growth factors, heat shock, hypoxia, chemokines and hyperosmolarity (Coffer et al.; Vanhaesebroeck and Alessi). It has been shown that PKBα is a regulator of cellular metabolic functions as well as apoptotic response (Coffer et al.). Since PKBα is an important regulator of cellular functions, we are investigating the possibility of improving transient transfection systems by over-expression of PKBα. We have examined three host cell lines, COS-1, CV-1EBNA (derivative of CV-1) and 293MSR (derivative of 293) by transient co-transfection of a reporter gene with a wild-type PKBα. Data suggest that all the host cells benefit by over-expression of PKBα as demonstrated by an improved recombinant protein titer. To determine whether the increased protein production is limited to transient co-transfection, stable clonal lines of CV-1EBNA and COS-1 cells that over-express PKBα were developed. We found that transient protein titers are improved when the hosts CV-1EBNA and COS-1 are stably over-expressing PKBα. The mechanism responsible for this improved performance is being investigated.
2. Materials and Methods

2.1. Tissue Culture

The 293MSR cells are a derivative of 293 human embryonic kidney cells that over-express the Epstein Barr Virus Nuclear Antigen-1 (EBNA) and macrophage scavenger receptor (MSR). The CV-1EBNA cell line is a derivative of the CV-1 fibroblast like African Green Monkey cell line (ATCC, Manassas, VA). CV-1EBNA cells constitutively express EBNA driven from the CMV immediate early enhancer/promoter. COS-1 is a cell line established from CV-1 cells which constitutively expresses Simian Virus 40 large T antigen (ATCC). CV-1EBNA and COS-1 cells are grown in Dulbecco’s Modified Eagles Medium/F12 (DMEM/F12 JRH, Lenexa, KS) with the addition of 1% penicillin streptomycin (100x stock GibcoBRL, Grand Island, NY), 1% Glutamax (GibcoBRL) and 5% fetal bovine serum (FBS) (Hyclone, Logan, VT). The 293MSR cells are grown in DMEM (Hyclone) with the addition of 1% penicillin streptomycin, 1% Glutamax and 10% FBS. For transient transfections, cell lines were transfected using standard DEAE/dextran (Sigma, St. Louis, MO) protocols which included a 10% dimethylsulfoxide (DMSO) (Sigma) shock and chloroquine (Sambrook et al.). The DMSO shock was not given to the 293MSR cells. Subsequent to the transfection, the cells were cultured in growth media containing low serum (0.5%). For co-transfection experiments, equal amounts of each plasmid were used. To create cell lines with stably integrated pcDNA3 (Invitrogen, Carlsbad, CA) or pcDNA3PKBα, COS-1 or CV-1EBNA cells were transfected with either plasmid using lipofectamine (GibcoBRL). Cells were selected in media containing G418 and G418 resistant cells were either pooled or plated in 96 well micro titer plates for individual cell cloning. Recombinant protein expression was monitored by ELISA or Western blotting. All expression studies were done at least twice in triplicate. Representative results are shown.

2.2. Plasmid Constructs

The reporter plasmids encoding Fc receptor 1, Fc receptor 2, Reporter 2 or a fluorescent reporter consisted of the cDNA for these reporters cloned into the expression plasmid pDC409 (Giri et al.). pDC409 includes the Epstein Barr Virus origin of replication and the SV40 Virus origin of replication. Expression of recombinant protein is driven by an expression cassette that includes the HIV trans-activator Tat driving the expression of the Tar promoter sequence. The reporter plasmid for Cytokine 1, pDC412 Cytokine 1, consisted of the cDNA for this protein cloned into pDC412. pDC412 expression plasmid is similar to pDC409, except that the multiple cloning site has been modified. Expression of Reporter 1 was driven by the SV40 promoter/enhancer. The cDNA for hamster PKBα was cloned using RT-PCR from Chinese hamster ovary cell RNA. An RT-PCR fragment-encoding hamster PKBα was cloned into the expression vector pcDNA3.

2.3. Western Blots

For Western blot analysis, cells were washed with PBS and lysed in cell lysis buffer (New England Biolabs, Beverly, MA). Lysates from equal numbers of cells were separated by