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Host cell targets of immediate-early protein BICP22 of bovine herpesvirus 1

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Abstract

The immediate-early (IE) protein BICP22 of bovine herpesvirus 1 (BHV-1) acts as transrepressor protein on viral promoters of different kinetic classes. In the present work, we looked for host cell targets of BICP22 using a yeast two-hybrid system and identified seven candidates: (1) JIK, a serine/threonine kinase of the sterile 20 protein (STE20) family that inhibits stress-related pathways; (2) cAMP response element binding protein-like 2 (CREBL2), which in its bZip domain shares homology with CREB, modulating transcription of cAMP responsive genes; (3) DNA-dependent ATPase and helicase (ATRX), a protein of the SNF2 family altering nucleosome structure; (4) scaffold attachment factor B (SAF-B), which helps to organize chromatin into topologically separated loops; (5) peptidylglycine alpha-amidating monooxygenase COOH-terminal interactor protein 1 (PAMCIP1), involved in regulation of the secretory pathway in the perinuclear area; (6) zinc finger protein (ZNF38) found in proliferating cells and possibly associated with meiosis in male and female gametogenesis; (7) FLJ22709, hypothetical protein conserved among various species, containing an occludin/ELL domain. To confirm some of the interactions by confocal fluorescence microscopy, BICP22 was tagged with red fluorescent protein in an amplicon, and selected target sequences were tagged with green fluorescent protein in plasmid expression vectors. Upon amplicon transduction of Vero cells and plasmid transfection, CREBL2 and ZNF38 both colocalized with BICP22 in distinct nuclear domains.

Keywords: Bovine herpesvirus 1; Immediate-early; Yeast two-hybrid; Amplicon; Nuclear protein

1. Introduction

Bovine herpesvirus 1 (BHV-1) causes an economically important disease of cattle (Engels and Ackermann, 1996; Schwyzer and Ackermann, 1996; Tikoo et al., 1995; Wyler et al., 1989), which is perpetuated in herds by latency, the hallmark of herpesviruses (Jones, 1999, 2003). The disease is also known as infectious rhinotracheitis/pustular vulvovaginitis (IBR/IPV), a respiratory or genital infection, often accompanied by abortions or neurological symptoms. During the lytic cycle of BHV-1 infection, viral genes are expressed in three temporal phases termed immediate-early (IE), early and late. Four IE proteins have been described, BHV-1 infected cell protein 0 (BICP0), BICP4, BICP22 and circ protein (Fraefel et al., 1994a; Schwyzer et al., 1993, 1994; Wirth et al., 1992). The

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best characterized IE protein is BICP0, a 97-kDa zinc finger protein that localizes predominantly to the nucleus and transactivates a variety of viral and cellular genes (Fraefel et al., 1994b; Zhang et al., 2005). Although not strictly essential, it is required for efficient virus production in cell culture (Köppel et al., 1996).

About BICP22, much less is known. Previous work (Köppel et al., 1997) showed that it is a predominantly nuclear protein of 50 kDa which acts as transrepressor protein on viral promoters of different kinetic classes. It does not affect its own mRNA, which is synthesized with dual kinetics, IE and late (Schwyzer et al., 1993). From its counterparts in other alphaherpesviruses, notably herpes simplex virus type 1 (HSV-1), varicella zoster virus, and equine herpesvirus 1, other potential properties of BICP22 can be inferred, such as phosphorylation on specific tyrosine residues affecting virulence (Brandt and Kolb, 2003; O'Toole et al., 2003), phosphorylation by UL13, a viral serine/threonine kinase (Hagglund et al., 2002; Kenyon et al., 2001), interaction with ICP4 (Derbigny et al., 2002; Lynch et al., 2002) and activation of caspase 3 (Hagglund et al., 2002).

The detailed mode of action of all BHV-1 IE proteins remains unknown, but as multifunctional viral proteins they are likely to interact with specific host cell proteins. Indeed, two different interactions of BICP0 with host cell targets have recently been demonstrated, one with histone deacetylase (Zhang and Jones, 2001) and the other with prostaglandin D synthase (Saydam et al., 2004). The latter interaction was discovered using the yeast two-hybrid system. In the present work, we screened a bovine cDNA library in a yeast two-hybrid system for potential host cell targets of BICP22 to understand better how BHV-1 exploits the host. Among the targets found, and deserving further scrutiny, four are bovine homologs of nuclear proteins with well-known functions, and one exhibits high sequence similarity to a putative zinc finger transcription factor.

2. Materials and methods

2.1. Yeast two-hybrid screening of cDNA library

The same bovine cDNA library that had been used for the screening of BICP0 (Saydam et al., 2004) was used here to find BICP22 targets. To construct a bait plasmid for BICP22, the EcoRV/DraI fragment (979 bp) of pCMV22AAA (Köppel et al., 1997) was inserted into pBTM116 (Saydam et al., 2004) cut with *Sma*I. The resulting plasmid pBTM116BICP22 encodes a fusion protein consisting of the LexA DNA binding domain fused to BICP22 (amino acids 1–300). Yeast transformations were carried out as described (Gietz et al., 1995). Cotransformants were plated on yeast dropout media lacking leucine, tryptophan and histidine (SD-TLH). Positive clones were identified by X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside) filter assay as described (Saydam et al., 2002).

2.2. Bait dependency test

All histidine and LacZ-positive yeast clones were tested for bait dependency (Saydam et al., 2004). From the clones, DNA was prepared as described (Hoffman and Winston, 1987) and then cotransformed individually with pBTM116 (empty vector) or pBTM116LaminC into the yeast strain L40. After 4–6 days of growth on SD-TLH plates, X-gal filter assay was performed. Clones staining white with empty vector and Lamin C were considered as bait-dependent. Clones showing blue staining either with empty vector or Lamin C were eliminated.

2.3. Sequence analysis, homology search and expression of fusion proteins

Bait-dependent bovine cDNA clones were sequenced (Microsynth, Balgach, Switzerland) using GAL855 forward screening primer (5′-TGT TTA ATA CCA CTA CAA TG-3′), and ADH termination reverse screening primer (5′-AAA TTG AGA TGG TGC ACG-3′). The GenBank database was searched for homologies (www.ncbi.nlm.nih.gov). To express a fusion protein with enhanced green fluorescent protein (eGFP) at the N-terminus, the bovine cAMP response element binding protein-like 2 (CREBL2) cDNA clone was cut with *Eco*RI/*Bgl*II and inserted into peGFP-C2 (Clontech) cut with *Eco*RI/*Bam*HI to give peGFP-CREBL2. Construction of peGFP-ZNF38 was analogous except that the plasmids were cut with *Eco*RI alone.