Journal of NeuroVirology, 15: 123–130, 2009
© 2009 Journal of NeuroVirology
ISSN 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280802534771

informa
healthcare

The cellular transcription factor, CCAAT enhancer–binding protein alpha (C/EBP-α), has the potential to activate the bovine herpesvirus 1 immediate-early transcription unit 1 promoter

Florencia Meyer, and Clinton Jones

Department of Veterinary and Biomedical Sciences, School of Biological Sciences, Nebraska Center for Virology, University of Nebraska, Lincoln, Nebraska, USA

Following acute infection, bovine herpesvirus-1 (BHV-1) establishes a lifelong latent infection in sensory neurons of trigeminal ganglia. BHV-1 periodically reactivates from latency and is shed as infectious virus. The latency-related (LR) gene is abundantly expressed in trigeminal ganglia of infected calves, and proteins encoded by the LR gene are necessary for reactivation from latency. We previously demonstrated that a novel LR protein interacts with a host transcription factor, CCAAT enhancer–binding protein alpha (C/EBPα). C/EBPα increases plaque-forming efficiency when cotransfected with BHV-1 DNA and its expression is induced in neurons during reactivation from latency (Meyer *et al*, 2007, *J Virol* 81: 59–67). The ability of C/EBPα to bind DNA is necessary for stimulating productive infection, suggesting C/EBPα stimulates viral transcription. We tested whether C/EBPα could *trans*-activate the BHV-1 immediate early transcription unit 1 (IEtu1) promoter because the IEtu1 promoter activates expression of two viral genes (bICP0 and bICP4) that stimulate producitve infection. In the current study, We demonstrate that C/EBPα and the BHV-1 *trans*-inducing factor (bTIF) synergistically *trans*-activate IEtu1 promoter activity. However, bICP0 and C/EBPα did not synergistically *trans*-activate IEtu1 promoter activity. Deletion of IEtu1 promoter sequences demonstrated that C/EBPα by itself could *trans*-activate a truncated IEtu1 promoter, suggesting sequences in the distal region of the IEtu1 promoter negatively regulate C/EBPα activtiy. These studies suggest that C/EBPα stimulates productive infection and reactivation from latency, in part, by cooperating with bTIF to activate IEtu1 promoter activity. *Journal of NeuroVirology* (2009) **15,** 123–130.

Keywords: bovine herpesvirus1; gene expression; C/EBP-alpha

Introduction

Bovine herpesvirus 1 (BHV-1) is an important pathogen that causes respiratory and genital disease, abortion, conjunctivitis, and multisystemic infection in neonate calves (Jones, 2007; Tikkoo, 1995). Like other members of the *alpha-herpesvirinae* subfamily, BHV-1 establishes latency in ganglionic neurons of the peripheral nervous system after initial infection of mucosal surfaces. Viral reactivation and spread to susceptible calves can occur after glucocorticoid-induced stress (Jones, 1998; Jones, 2003).

Viral gene expression is temporally regulated in three distinct phases: immediate early (IE), early (E), or late (L). IE transcription unit 1 (IEtu1) encodes a functional homolog of two HSV-1 proteins, ICP0 and

Address correspondence to Clinton Jones, Department of Veterinary and Biomedical Sciences, School of Biological Sciences, Nebraska Center for Virology, University of Nebraska, Lincoln, NE 68503, USA. E-mail: cjones@unlnotes.unl.edu

This work was primarily supported by two USDA grants (2008–00891 and 2006–01627). A NIAID grant to C.J. (R21AI069176) and a COBRE grant to the NE Center for Virology (1P20RR15635) also supported certain aspects of this study.

Received 24 July 2008; revised 3 September 2008; accepted 6 October 2008

ICP4. BHV-1–encoded ICP0 (bICP0) is important for productive infection because it activates all viral promoters, and is expressed at high levels throughout infection (Fraefel *et al*, 1994; Wirth *et al*, 1989, 1991, 1992). The BHV-1 *trans*-inducing factor (bTIF) selectively activates IE promoters by interacting with cellular proteins and viral DNA sequences that are only detected in BHV-1 IE promoters (Lu and Misra, 2000a,b; Misra *et al*, 1994, 1995, 1996). The bTIF protein is present in the viral tegument and consequently readily activates IE RNA expression in the absence of protein expression.

During latency, viral gene expression is restricted to the latency-related (LR) gene (Rock and Mayfield, 1987) and open reading frame (ORF)-E gene (Inman, 2004). The LR gene encodes at least three proteins (Hossain *et al*, 1995; Jiang, 2004; Meyer, 2007) whose expression correlates with the ability of BHV-1 to reactivate from latency following dexamethasone treatment (Inman, 2001; Inman, 2002). LR RNA is alternatively spliced in bovine trigeminal ganglia (TG) compared to non-neural cells (Devireddy and Jones, 1998; Hossain *et al*, 1995). Alternatively spliced LR transcripts are detected in TG at 1, 7, 15, or 60 days post infection (Devireddy and Jones, 1998). A polyA+ spliced LR RNA is abundantly expressed at 7 days post infection (d.p.i.) in TG of infected calves. Splicing results in an in-frame fusion of the two major LR open reading frames, ORF2 and ORF1 (Devireddy *et al*, 2003; Devireddy and Jones, 1998), which can be translated into a protein (Jiang, 2004). A bacterial two-hybrid screen identified several proteins, including the CCAAT enhancer–binding protein alpha (C/EBPα), that interact with the novel LR protein (Meyer, 2007). C/EBPα expression is induced in sensory neurons of latently infected calves during reactivation from latency, and increases plaque forming efficiency (Meyer, 2007). These studies suggested that C/EBPα activates productive infection, whereas C/EBPα interactions with a LR protein inhibit productive infection and promote establishment of latency.

C/EBPα belongs to a family of basic region leucine zipper class DNA-binding proteins (Wedel, 1995). High conservation in the basic and leucine zipper domains allows for heterodimer formation between all family members (Ramji, 2002). The N-terminal sequences of C/EBPα contain amino acid sequences that promote cooperative binding to the TATA box–binding protein and TFIIB (Transcription factor II B), as well as proteins that negatively regulate transcription (Lekstrom-Himes, 1988; Nerlov, 1995). C/EBPα regulates the cell cycle by interacting with and inhibiting cyclin-dependent kinases 2 (cdk2) and cdk4 (Wang, 2001; Wang, 2002; Wang, 2005). The human and mouse Bim promoter contain a consensus C/EBPα binding site, which is activated by an endoplasmic reticulum stress-induced transcription factor (CHOP) and C/EBPα (Puthalakath, 2007). Bim is a Bcl-2 family member that regulates endoplasmic reticulum stress-induced apoptosis (Puthalakath, 2007). These studies and others demonstrate that C/EBPα regulates differentiation, metabolism, inflammation, and cell death (Ramji, 2002). C/EBPα also stimulates lytic gene expression of several gammaherpesvirus, Epstein-Barr virus (EBV), human herpesvirus 8 (HHV-8), and herpesvirus saimiri (HVS), for example (Huang, 2006; Wakenshae, 2005; Wang, 2003a; Wu, 2004).

In this study we demonstrated that C/EBPα and bTIF synergistically activate the BHV-1 IETu1 promoter. The ability of C/EBPα to bind DNA was necessary for synergistic activation. In contrast, bICP0 and C/EBPα did not synergistically *trans*-activate the IEtu1 promoter. As judged by chromatin immunoprecipitation (ChIP) assays, we found that C/EBPα was associated with the IETu1 promoter during productive infection. Finally, C/EBPα by itself *trans*-activated an IEtu1 promoter containing a 1300-bp deletion from the 5′ terminus, suggesting negative regulatory elements exist in the IEtu1 promoter.

Results

C/EBPα modulates viral promoter activity

A previous study demonstrated that C/EBPα stimulates productive infection and C/EBPα DNA-binding activity is necessary (Meyer, 2007), suggesting C/EBPα *trans*-activates viral promoters. Consequently, we tested whether C/EBPα activates the IEtu1 promoter because this promoter controls expression of two important viral transcripitonal regulators (bICP0 and bICP4) (Figure 1A). A CAT expression plasmid containing the IETu1 promoter (Figure 1B) was cotransfected with a plasmid that expresses wild-type (wt) C/EBPα or mutant (mut) C/EBPα. In 9.1.3 bovine cells or mouse neuroblastoma cells (neuro-2A), the IETu1 promoter was not stimulated by wt C/EBPα alone (Figure 2A and B).bTIF and bICP0 can *trans*-activate the IETu1 promoter (Fraefel *et al*, 1994; Misra *et al*, 1994; Wirth *et al*, 1991), and thus were used as positive controls. Under the conditions of this study, bTIF stimulated IETu1 promoter activity 2-fold in neuro-2A or 9.1.3 cells, and bICP0 activated IEtu1 promoter activity 5- to 6-fold (Figure 2A and B). A synergistic activation of IEtu1 promoter activity was observed when bTIF and wt C/EBPα were used to cotransfect neuro-2A or 9.1.3 cells (Figure 2A). bTIF activation increased from 2- to 20–25-fold when wt C/EBPα was present. In the case of bICP0, wt C/EBPα enhanced *trans*-activation from 5- to 11-fold, suggesting an additive effect (Figure 2B). The ability of C/EBPα to bind DNA was required because transfection of the DNA-binding mutant (mut C/EBPα) did not enhance *trans*-activation of the IEtu1 promoter by bTIF or bICP0.

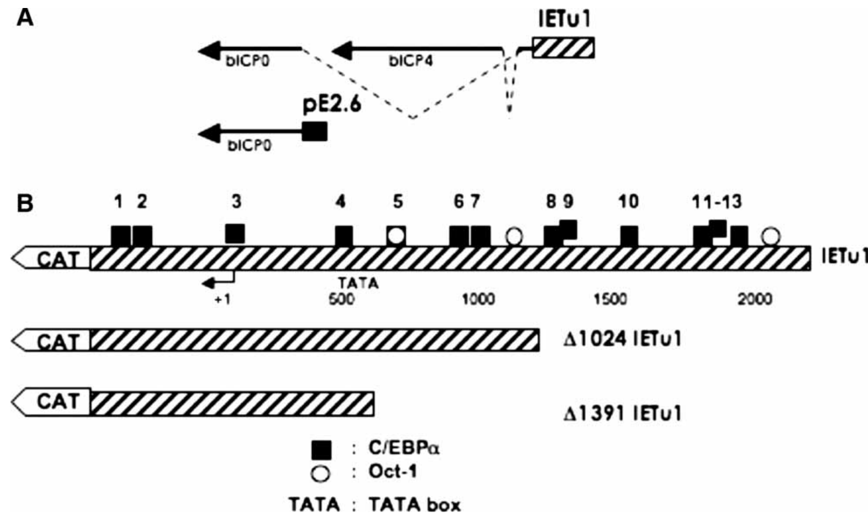

Figure 1 Diagram of immediate-early and early promoters. (**A**) The IETu1 promoter drives expression of bICP4 and bICP0, whereas an early promoter (pE2.6) controls expression of bICP0 after IETu1 is shut off by bICP22 and bICP4 (Wirth *et al*, 1992). A solid arrow denotes the transcripts from the IEtu1 and E2.6 promoters. (**B**) A scheme of the two IETu1 deletion constructs (Δ1024 IETu1 and Δ1391 IETu1) used in this study. The positions of the putative C/EBPα binding sites (*black bars*), two Oct-1 binding sites (*open circles*), and the TATA boxes are shown. Position of the CAT (chloramphenicol acetyltransferase) gene at the 3' terminus of the IEtu1 promoter constructs is also shown.

The ability of C/EBPα to enhance transcription was also examined using the HSV-1 VP16 gene, which is the functional homologue of bTIF. Although VP16 activated the BHV-1 IETu1 in a similar fashion as bTIF in neuro-2A cells, C/EBPα did not have a dramatic effect on promoter activity (Figure 3). These studies suggested that C/EBPα and bTIF are specific to BHV-1, but had no effect on the *trans*-activation potential of HSV-1–encoded VP16.

Localization of IETu1 promoter sequences that are responsive to bTIF and C/EBPα

To localize IEtu1 promoter sequences that are necessary for synergistic *trans*-activation by bTIF and C/EBPα, two deletion promoter constructs that lack the 5′ terminal 1024 bp (Δ1024IETu1) or the 5′ terminal 1391 bp (Δ1391IETu1) were analyzed (see Figure 1B for schematic of the IEtu1 deletion constructs). Deletion of these sequences did not dramatically change the basal promoter activity of these deletion constructs relative to the full-length IEtu1 promoter (data not shown). bTIF activated the Δ1024IETu1 construct approximately 4-fold in neuro-2A cells (Figure 4A). Activation of Δ1391IETu1 by bTIF was approximately 3-fold (Figure 4B). Cotransfection of wt C/EBPα and bTIF enhanced *trans*-activation of Δ1024IETu1 from 4- to 18-fold, and Δ1391IETu1 from 3- to 25-fold, which was similar to that observed with the full-length promoter (Figure 2).

Surprisingly, wt C/EBPα, but not the C/EBPα mut construct, *trans*-activated Δ1024IETu1 promoter activity approximately 4-fold (Figure 4A). C/EBPα *trans*-activated the Δ1391IETu1 promoter construct nearly 13-fold. These results indicated that C/EBPα efficiently *trans*-activated the IEtu1 promoter when 1391 bp was removed from the 5′ terminus.

C/EBPα interacts with the IETu1 promoter during productive infection

If C/EBPα has a direct effect on IEtu1 promoter activity, we predict that C/EBPα interacts with IETu1 promoter sequences because the DNA-binding activity of C/EBPα was important for stimulating IEtu1 promoter activity (Figures 2–4). The full-length IEtu1 promoter contains 13 putative C/EBPα binding sites that were identified by computer analysis (AliBaba2.1, 2000) (Figure 1B). An Oct-1 binding site that is similar, but not identical, to the putative C/EBPα binding site 5 was also identified in the IEtu1 promoter (Figure 1B).

To examine whether C/EBPα interacts with the IETu1 promoter, chromatin immunoprecipitation (ChIP) assays were performed following infection of MDBK cells with BHV-1 using an MOI of 1. At 5 or 6 h after infection, C/EBPα was associated with the IEtu1 promoter because an amplified band was detected following IP of chromatin with the C/EBPα antibody (Ab) (Figure 5A). In contrast, this amplified band was absent or less intense in the no Ab control (Figure 5A). As expected, we were not able to amplify the IEtu1 promoter in mock-infected cells (Figure 5B). Primers encompassing the bICP0 ORF (bICP0 ORF 5′) amplified a band in the no antibody lane (no Ab) with similar efficiency as the band obtained following IP with the C/EBPα antibody (Figure 5C, Ab lane), suggesting that C/EBPα does not specifically bind to the bICP0 ORF. Furthermore, C/EBPα does not specifically bind to the BHV1 TK promoter because we were unable to amplify the expected product

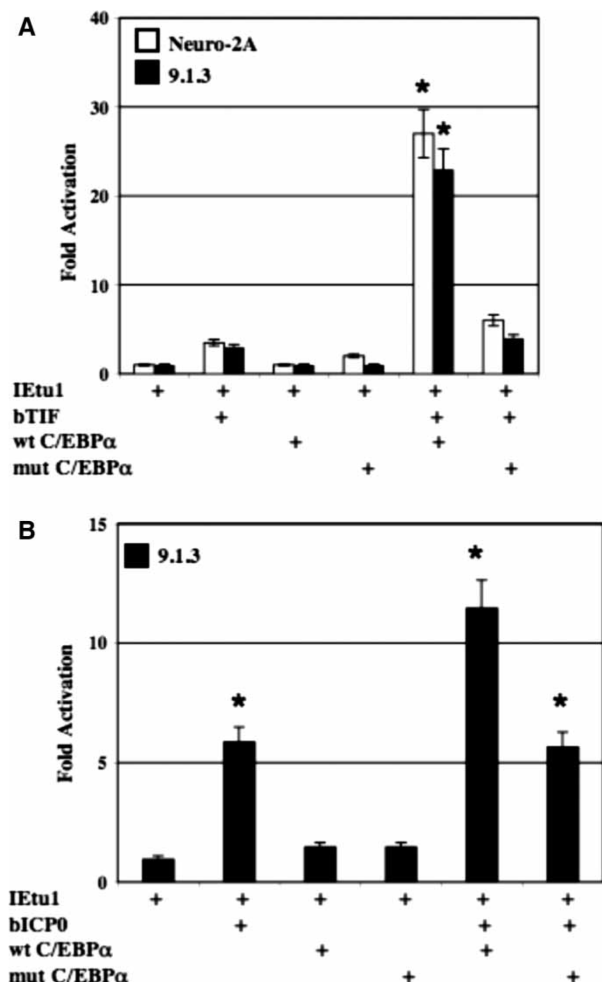

Figure 2 C/EBPα enhances activity of viral transactivators. Neuro-2A or 9.1.3 cells were cotransfected with 1 μg of the full-length IETu1 promoter CAT construct and the indicated combination of plasmids. For these studies, 1 μg of wt or mut C/EBPα plasmids and either 4 μg of bTIF or 1 μg of bICP0 were used. The total amount of transfected DNA was identical for all transfections because the empty expression vector (pcDNA3.1) was added to the mixture. At 48 h post transfection, cell lysate was collected and assayed for CAT activity. CAT activity of cells transfected only with the IETu1 promoter was set to 1-fold. All other values are expressed as fold activation with respect to the control. (**A**) The viral activator used was bTIF. $*P<.01$ when comparing the second to the fifth column. (**B**) The viral activator used was bICP0. $*P<.05$ when comparing to basal promoter activity of IEtu1. **A** and **B** show the average of at least three independent experiments. Statistical analysis was performed using single-factor ANOVA.

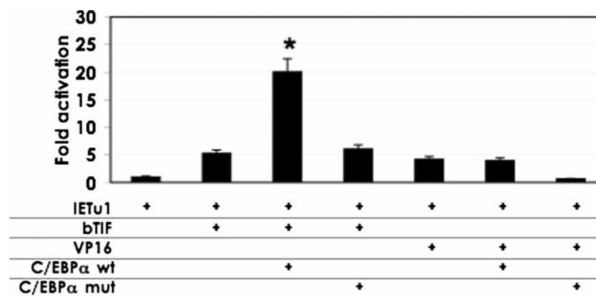

Figure 3 VP16 and C/EBPα do not synergistically activate the IEtu1 promoter. Similar experiment as is Figure 2 using the IETu1 promoter and bTIF (4 μg) or the HSV-1 VP16 homolog (3.5 μg) as activators. $*P<.05$ when comparing to all of the other samples. **A** and **B** show the average of at least three independent experiments. Statistical analysis was performed using single-factor ANOVA.

following IP with the C/EBPα antibody (Figure 5C). In summary, these studies suggested that C/EBPα was associated with the IETu1 promoter during productive infection.

Discussion

In this study, we demonstrated that C/EBPα and bTIF synergistically activate IETu1 promoter activity. The ability of C/EBPα to interact with IEtu1 promoter sequences correlated with activation. A previous study demonstrated that C/EBPα protein levels increase during BHV-1 productive infection and reactivation from latency (Meyer, 2007). C/EBPα also stimulates plaque formation following transfection of BHV-1 DNA into bovine cells (Meyer, 2007). The ability of C/EBPα to synergistically cooperate with bTIF to activate IEtu1 promoter activty would activate productive infection because this promoter drives expression of bICP0 and bICP4. We suggest that bTIF and C/EBPα activate IEtu1 promoter activity during acute infection because bTIF is encoded by a late gene (Misra *et al*, 1994) and thus would not be abundantly expressed during the early phase of reactivation from latency.

C/EBPα may have a direct effect on IEtu1 promoter activity because C/EBPα interacted with IEtu1 promoter sequences during productive infection (Figure 5), and the ability of C/EBPα to bind DNA was necessary for transcriptional activation (Figures 2–4). The interactions between C/EBPα and IEtu1 promoter sequences may not be stable or may require multiple C/EBPα binding sites because we were unable to demonstrate binding when C/EBPα was overexpressed in 293 cells using gel shift assays and single C/EBPα binding sites (Meyer and Jones, unpublished data). It is also possible that viral-encoded or -induced proteins stabilize interactions between IEtu1 promoter sequences and C/EBPα.

Following deletion of 1024 or 1391 bp from the full-length IEtu1 promoter, C/EBPα *trans*-activated this promoter in the absence of bTIF (Figure 4), suggesting that sequences in the full-length IEtu1 promoter inhibited C/EBPα *trans*-activation unless bICP0 or bTIF was present. These deleted sequences may contain binding sites for proteins that repress transcription, or this region may exist in a chromatin structure that inhibits C/EBPα-induced *trans*-activation following transfection of plasmids containing the full-length IEtu1 promoter into cultured cells. Because C/EBPα protein and RNA expression is stimulated during dexamethasone-induced BHV-1

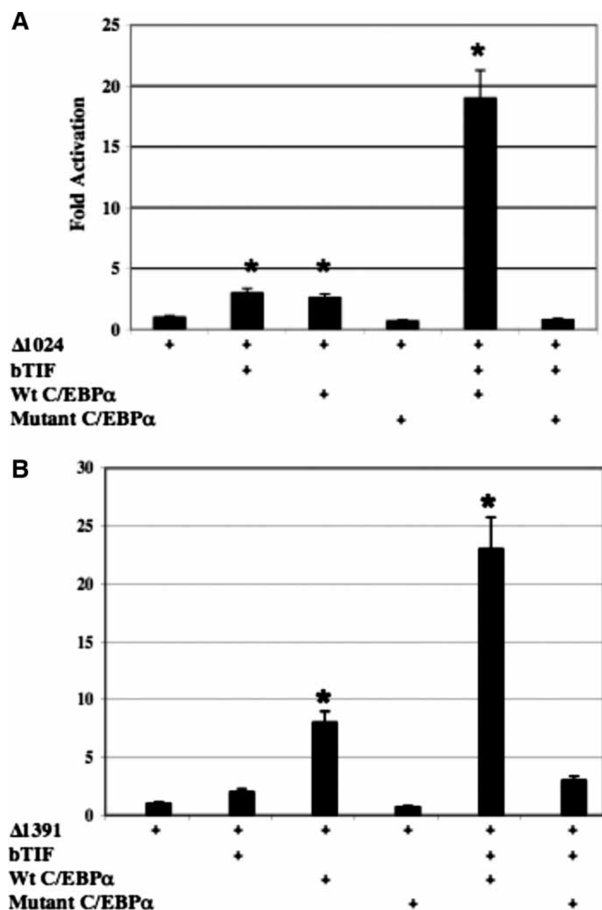

Figure 4 Localization of IETu1 promoter sequences that are important for *trans*-activation. Bovine 9.1.3 cells were cotransfected with1 μg of Δ1024 IETu1 (**A**) or Δ1931 IETu1 (**B**) promoter constructs driving expression of the CAT reporter gene and 1 μg of each of the indicated combination of plasmids. DNA amounts were equalized for all transfections by adding the necessary empty expression vector pcDNA3.1. At 48 h post transfection, cell lysate was collected and processed for CAT activity. CAT activity of the cells transfected only with the IETu1 promoter (first column) was the control and set to 1 fold. All other values are expressed as fold activation with respect to the control. Experiments in **A** and **B** constitute the average of three independent experiments. $^*P < .05$ when comparing to basal promoter activity in lane of panel A and B. Statistical analysis was performed using single-factor ANOVA.

reactivation from latency (Meyer, 2007), we speculate that C/EBPα may also activate other viral promoters during reactivation from latency. Studies to address which viral genes are activated during reactivation from latency and whether C/EBPα activates the promoters of these genes are currently underway.

Materials and methods

Cells

Bovine epidermal cells (9.1.3), murine neuroblastoma 2A cells (neuro-2A), and human AD-293 cells

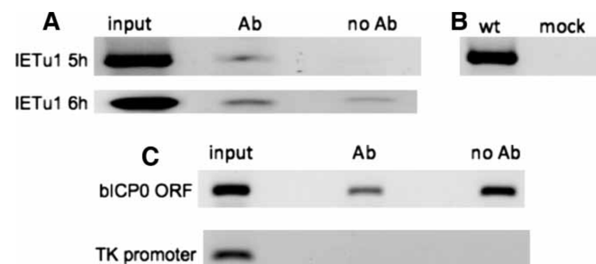

Figure 5 C/EBPα interacts with IETu1 promoter sequences during productive infection. MDBK cells were infected at an MOI of 1 for 5 or 6 h. Cells were then lysed, cross-linked, and sonicated as described in Materials and Methods. An aliquot of this material was kept (input) and the rest divided in two tubes, one of which was immunoprecipitated with 20 μg of a C/EBPα antibody (Ab) (Santa Cruz). The other tube received no antibody (no Ab). The antibody was collected with protein A agarose beads and the DNA that was immunoprecipitated was used for PCR to amplify a region that overlaps the TATA box in IEtu1 (**A**). (**B**) The same IEtu1 primers were shown to amplify total chromatin from cells infected with BHV-1, but not from mock-infected cells. The IE5F and IE4R primer pairs were used to amplify the IEtu1 promoter. (**C**) (*Top*) MDBK cells were infected with wt BHV-1 for 5 h, and ChIP analysis performed using primers that amplify the bICP0 ORF (bICP0 ORF 5' or bICP0 PII). (*Bottom*) TK promoter primers were also used to test whether C/EBPα was associated with TK promoter sequences. All primers were described in Materials and Methods. These results are representative of four independent studies.

were grown in Earle's modified Eagle's medium (EMEM) supplemented with 10% fetal calf serum (FCS). Bovine kidney cells (MDBK) were grown in EMEM supplemented with 5% FCS. All media contained penicillin (10 U/ml) and streptomycin (100 μg/ml).

Plasmids

The C/EBPα wild-type (wt) plasmid contains the mouse C/EBPα in an adenoviral vector (pAdTrack), which allows for efficient expression in transient transfection experiments (Wang, 2003b). The C/EBPα mutant (mut) plasmid contains a single amino acid change (R290A) in C/EBPα, which results in loss of DNA-binding activity (Wang, 2005).

bTIF contains all of the coding sequence of the BHV-1 bTIF gene regulated by the cytomegalovirus IE promoter in the pcDNA backbone (Invitrogen) (Misra *et al*, 1994). The bICP0 expression plasmid contains the bICP0 coding sequences under the control of the cytomegalovirus IE promoter and an N-terminus flag-tag (Zhang, 2006).

IETu1-CAT contains IETu1 promoter sequences that were cloned upstream of pSV0CAT (a promoter minus chloramphenicol acetyltransferase [CAT] expression vector), and this plasmid was provided by V. Misra (Saskatoon, Canada). Two deletion constructs, Δ1024 IETu1 and Δ1391 IETu1 have 1024 or 1391 bp removed from the 5' terminus, respectively (Figure 1B). The empty vector pcDNA3.1 was purchased from Invitrogen.

Chloramphenicol acetyltransferase (CAT) reporter assays

Cells grown in 60-mm-diameter dishes were transfected with an IETu1 reporter construct and bICP0 (or mutants derived from bICP0), bTIF, C/EBPα wt, or C/EBPα mut. The combination and amounts of DNA used are described in the figure legends. 9.1.3 cells were transfected with Lipofectamine 2000 (Invitrogen) and neuro-2A cells with TransIT (Mirus), according to the manufacturer's instructions. At the designated time after transfection, cell extract was prepared by three freeze/thaw cycles in 0.25 M Tris-HCl, pH 8.0. CAT activity was measured in the presence of 0.1 μCi [^{14}C]chloramphenicol and 0.5 mM acetyl coenzyme A (CoA) (Sigma). Incubation times varied from 1 to 5 h, depending on the promoter and cell line used. For a 5-h reaction, half the amount of acetyl CoA was added at the start point and the rest after 2.5 h. All forms of chloramphenicol were separated by thin-layer chromatography. CAT activity in cell extract containing 20 μg of protein was quantified using a Bio-Rad Molecular Imager FX. Levels of CAT activity are expressed as fold induction relative to the vector control.

Chromatin immunoprecipitation (ChIP) assay

Approximately 8 x 10^7 MDBK cells were infected with BHV-1 (Cooper strain) using an multiplicity of infection (MOI) of 1 plaque-forming unit/cell, and cells were collected at 5 or 6 h after infection. Cells were washed with phosphate-buffered saline (PBS) and suspended in 50 ml of medium with no serum. A volume of 1.35 ml of 37% formaldehyde was added for cross-linking and the cell suspension was allowed to gently shake at 20°C for 15 min. Cross-linking was stopped by addition of 2.5 ml of 2.5 M glycine and then incubating at 4°C for 5 min. Cells were pelleted by centrifuging at $1000 \times g$ followed by two washes with ice-cold PBS that contained 1 mM phenylmethylsulfonyl sluoride (PMSF). The final pellet was suspended in 3 ml of cell lysis buffer (5 mM PIPES pH 8, 85 mM KCl, 0.5% Nonidet P40 [NP40]) and incubated on ice for 10 min. Cells were vortexed every 2 min to promote lysis. Crude nuclei were pelleted and suspended in 3 ml of nuclear lysis buffer (50 mM Tris-HCl pH 8.1, 10 mM EDTA, 1% sodium dodecyl sulfate [SDS]) and incubated on ice for 10 min. The suspension was then sonicated three times for 30 s on ice. Sonicated samples were divided into two tubes and diluted to 10 ml with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, pH 8, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl, 1 mM PMSF). Samples were precleared by adding 75 μl of agarose/salmon sperm DNA protein A beads (Upstate) and incubating for 1 h at 4°C. Agarose beads were removed by centrifugation and 10 μg of C/EBPα antibody (Ab) was added. A tube that contained no antibody is referred to as the no Ab control. Tubes were incubated overnight at 4°C, and samples were continuously rotated. Seventy-five microliters of agarose protein A beads were added the next morning and allowed to incubate at 4°C. Beads were pelleted and washed with 1 ml of each of the following buffers: low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, pH 8, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, pH 8, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% NP40, 1% sodium deoxycholate, 1 mM EDTA, pH 8), and TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). DNA-protein complexes were eluted from beads by incubating with 500 μl of elution buffer (1% SDS, 0.1 M $NaHCO_3$) and vortexing gently for 15 min at room temperature. Agarose beads were centrifuged and the supernatant transferred to another tube. Twenty microliters of 5 M NaCl was added to each tube and placed in a water bath at 65°C overnight to de-cross-link proteins from DNA. Samples were then extracted once with phenol:-chlorophorm:isoamyl alcohol and once with chloroform. DNA was precipitated with isoamyl alcohol, washed with 70% ethanol, dried in a vacuum microfuge, and suspended in 30 to 50 μl of water. Polymerase chain reaction (PCR) was then performed using primers described below.

The IE5F primer (cggccatgctttcatgcaaatgagccccgacagcc) and the IE4R primer (AGCAGCGGCAGCGGCAGGTGTTGCAGTACGGGTGT) amplify a 255-bp fragment that overlaps the TATA box of the IETu1 promoter. The bICP0 ORF 5' primers amplify a region of the bICP0 ORF and these primers are + CCGTGCAGTCTCTCATCCACA and -GGTCAATAAACTCCTGCGCCGCGT. The TK primers amplify a region of the TK promoter and they are + GCCGCCGTACTGGACATGCG and -GCCGAGTCCCCGTAAGGCGAT. All primers are listed as 5' to 3'.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Devireddy L, Zhang Y, Jones C (2003). Cloning and initial characterization of an alternatively spliced transcript encoded by the bovine herpes virus 1 latency related (LR) gene. *J NeuroVirol* **9:** 612–622.

Devireddy L, and C Jones (1998). Alternative splicing of the latency-related transcript of bovine herpesvirus 1 yields RNAs containing unique open reading frames. *J Virol* **72:** 7294–7301.

Fraefel C, Zeng J, Choffat Y, Engels M, Schwyzer M, Ackermann M (1994). Identification and zinc

dependence of the bovine herpesvirus 1 transactivator protein BICP0. *J Virol* **68:** 3154–3162.

Hossain A, LM Schang and C Jones (1995). Identification of gene products encoded by the latency-related gene of bovine herpesvirus 1. *J Virol* **69:** 5345–5352.

Huang J, G Liao, H Chen, FY Wu, L Hutt-Fletcher, GS Hayward, and SD Hayward (2006). Contribution of the C/EBP proteins to Epstein-Barr virus lytic gene expression and replication in epithelial cells. *J Virol* **80:** 1098–1109.

Inman M, L Lovato, A Doster, and C Jones (2001). A mutation in the latency-related gene of bovine herpesvirus 1 leads to impaired ocular shedding in acutely infected calves. *J Virol* **75:** 8507–8515.

Inman M, L Lovato, A Doster, and C Jones (2002). A mutation in the latency related gene of bovine herpesvirus 1 interferes with the latency-reactivation cycle of latency in calves. *J Virol* **76:** 6771–6779.

Inman M, J Zhou, H Webb, and C Jones (2004). Identification of a novel transcript containing a small open reading frame that is expressed during latency, and is antisense to the latency related gene of bovine herpes virus 1 (BHV-1). *J Virol* **78:** 5438–5447.

Jiang Y, M Inman, Y Zhang, NA Posadas, and C Jones (2004). A mutation in the latency related gene of bovine herpesvirus 1 (BHV-1) inhibits protein expression of a protein from open reading frame 2 (ORF-2) and an adjacent reading frame during productive infection. *J Virol* **78:** 3184–3189.

Jones C (1998). Alphaherpesvirus latency: its role in disease and survival of the virus in nature. *Adv Virus Res* **51:** 81–133.

Jones C (2003). Herpes simplex virus type 1 and bovine herpesvirus 1 latency. *Clin Micro Rev* **16:** 79–95.

Jones C, and S Chowdhury (2007). A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex, and development of improved vaccines. *Adv in Anim Health* **8:** 187–205.

Lekstrom-Himes J, and KG Xanthopoulos (1988). Biological role of the CCAAT/enhancer binding protein family of transcription factors. *J Biol Chem* **273:** 28545–28548.

Lu R, and V Misra (2000a). Potential role for luman, the cellular homologue of herpes simplex virus VP16 (alpha gene trans-inducing factor), in herpesvirus latency. *J Virol* **74:** 934–943.

Lu R, and V Misra (2000b). Zhangfei: a second cellular protein interacts with herpes simplex virus accessory factor HCF in a manner similar to Luman and VP16. *Nucleic Acids Res* **28:** 2446–2454.

Meyer F, S Perez, V Geiser, M Sintek, M Inman, and C Jones (2007). A protein encoded by the bovine herpes virus 1 (BHV-1) latency related gene interacts with specific cellular regulatory proteins, including the CCAAT enhancer binding protein alpha (C/EBP-a). *J Virol* **81:** 59–67.

Misra V, AC Bratanich, D Carpenter, P O'Hare (1994). Protein and DNA elements involved in transactivation of the promoter of the bovine herpesvirus (BHV) 1 IE-1 transcription unit by the BHV alpha gene trans-inducing factor. *J Virol* **68:** 4898–4909.

Misra V, S Walker, S Hayes, P O'Hare (1995). The bovine herpesvirus alpha gene trans-inducing factor activates transcription by mechanisms different from those of its herpes simplex virus type 1 counterpart VP16. *J Virol* **69:** 5209–5216.

Misra V, S Walter, P Yang, S Hayes, P O'Hare (1996). Conformational alteration of Oct-1 upon DNA binding dictates selectivity in differential interactions with related transcriptional coactivators. *Mol Cell Biol* **16:** 4404–4413.

Nerlov CA, and EB Ziff (1995). CCAAT/enhancer binding protein-alpha amino acid motifs with dual TBP and TFIIB binding ability co-operate to activate transcription in both yeast and mammalian cells. *EMBO J* **14:** 4318–4328.

Puthalakath H, LA O'Reilly, P Gunn, L Lee, PN Kelly, ND Huntington, PD Hughes, EM Michalak, J McKrimm-Breschkin, N Motoyama, T Gotch, S Akira, P Bouillet, and A Strasser. (2007). ER stress triggers apoptotis by activating BH3-only protein Bim. *Cell* **129:** 1337–1349.

Ramji DP and P. Foka (2002). CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem J* **365:** 561–675.

Rock DL, SL Beam, and JE Mayfield (1987). Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. *J Virol* **61:** 3827–31.

Tikkoo SK, M Campos, and LA Babiuk (1995). Bovine herpesvirus 1 (BHV-1): Biology, pathogenesis and control. *Virus Res* **45:** 191–223.

Wakenshae L, MS Walters, and A Whitehouse (2005). The herpesvirus saimiri replication and transcription activator acts synergistically with CCAAT enhancer binding protein alpha to activate the DNA polymerase promoter. *J Virol* **79:** 13548–13560.

Wang G, T Goode, P Iakova, JH Albrecht, and NA Timchenko (2002). C/EBPalpha triggers proteasome-dependent degradation of cdk4 during growth arect. *EMBO J* **21:** 930–941.

Wang G and NA Timchenko (2005). Dephosphorylated C/EBPalpha accelerated cell proliferation through sequestering retinoblastoma protein. *Mol Cell Biol* **25:** 1325–1338.

Wang GPI, M Wilde, A Welm, T Goode, WJ Roesler, and NA Timchenko (2001). C/EBPalpha arrests cell proliferation through direct interaction of Cdk2 and Cdk4. *Mol Cell* **8:** 817–828

Wang SE, FY Wu, Y Yu, and GS Hayward (2003a). CCAAT/enhancer-binding protein-alpha is induced during the early stages fo Kaposi's sarcoma-associated herpesvirus (KSHV) lytic cycle reactivation and together with the KSHV replication and transcription activator (RTA) cooperatively stimulates the viral RTA, MTA, and PAN promoters. *J Virol* **77:** 9590–9612.

Wang SE, FY Wu, Y Yu, and GS Hayward. (2003b). CCAAT/enhancer-binding protein-alpha is induced during the early stages of Kaposi's sarcoma-associated herpesvirus (KSHV) lytic cycle reactivation and together with the KSHV replication and transcription activator (RTA) cooperatively stimulates the viral RTA, MTA, and PAN promoters. *J Virol* **17:** 9590–9612.

Wedel A, and HW LomsZeigler-Heitbrock (1995). The C/EBP family of transcription factors. *Immunolobiology* **193:** 171–185.

Wirth UV, C Fraefel, B Vogt, C Vlcek, V Paces, M Schwyzer (1992). Immediate-early RNA 2.9 and early RNA 2.6 of bovine herpesvirus 1 are 3' coterminal and encode a putative zinc finger transactivator protein. *J Virol* **66:** 2763–2772.

Wirth UV, K Gunkel, M Engels, M Schwyzer (1989). Spatial and temporal distribution of bovine herpesvirus 1 transcripts. *J Virol* **63:** 4882–4889.

Wirth UV, B Vogt, M Schwyzer (1991). The three major immediate-early transcripts of bovine herpesvirus 1 arise from two divergent and spliced transcription units. *J Virol* **65:** 195–205.

Wu FY, SE Wang, H Chen, L Wang, SD Hayward, and GS Hayward (2004). CCAAT/enhancer binding protein alpha binds to the Epstein-Barr virus (EBV) ZTA protein through oligomeric interactions and contributes to cooperative transcriptional activation of the ZTA promoter through direct binding to the ZII and ZIIIB motifs during productive infection of the EBV lytic cycle. *J Virol* **78:** 4847–4865.

Zhang Y, Y Jiang, J Zhou, and C Jones. (2006). The bovine herpes virus 1 (BHV-1) immediate early protein (bICP0) interacts with the histone acetyltransferase p300, and these interactions correlate with stimulation of gC promoter activity. *J Gen Virol* **87:** 1843–1851.

This paper was first published online on iFirst on 24 December 2008.