Published in final edited form as:

Virus Res. 2011 September ; 160(1-2): 143–149. doi:10.1016/j.virusres.2011.06.003.

Cytoplasmic localized infected cell protein 0 (bICP0) encoded by bovine herpesvirus 1 inhibits beta interferon promoter activity and reduces IRF3 (interferon response factor 3) protein levels

Leticia Frizzo da Silva^{1,2}, Natasha Gaudreault^{2,3}, and Clinton Jones^{1,2,3,*}

¹ School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, Lincoln, NE 68503, Fair Street at East Campus Loop, Lincoln, NE, 68583-0905

² Nebraska Center for Virology, University of Nebraska, Lincoln, NE 68503, Fair Street at East Campus Loop, Lincoln, NE, 68583-0905

³ School of Biological Sciences, University of Nebraska, Lincoln, NE 68503, Fair Street at East Campus Loop, Lincoln, NE, 68583-0905

Abstract

Bovine herpesvirus 1 (BHV-1), an alpha-herpesvirinae subfamily member, establishes a life-long latent infection in sensory neurons. Periodically, BHV-1 reactivates from latency, infectious virus is spread, and consequently virus transmission occurs. BHV-1 acute infection causes upper respiratory track infections and conjunctivitis in infected cattle. As a result of transient immunosuppression, BHV-1 infections can also lead to life-threatening secondary bacterial pneumonia that is referred to as bovine respiratory disease. The infected cell protein 0 (bICP0) encoded by BHV-1 reduces human beta-interferon (IFN- β) promoter activity, in part, by inducing degradation of interferon response factor 3 (IRF3) and interacting with IRF7. In contrast to humans, cattle contain three IFN- β genes. All three bovine IFN- β proteins have anti-viral activity; but each IFN- β gene has a distinct transcriptional promoter. We have recently cloned and characterized the three bovine IFN- β promoters. Relative to the human IFN- β promoter, each of the three IFN- β promoters contain differences in the four positive regulatory domains that are required for virus-induced activity. In this study, we demonstrate that bICP0 effectively inhibits bovine IFN- β promoter activity following transfection of low passage bovine cells with interferon response factor 3 (IRF3) or IRF7. A bICP0 mutant that localizes to the cytoplasm inhibits bovine IFN- β promoter activity as efficiently as wt bICP0. The cytoplasmic localized bICP0 protein also induced IRF3 degradation with similar efficiency as wt bICP0. In summary, these studies suggested that cytoplasmic localized bICP0 plays a role in inhibiting the IFN- β response during productive infection.

© 2011 Elsevier B.V. All rights reserved.

*Corresponding Author cjones@unlnotes.unl.edu, Phone: (402) 472-1890, FAX: (402) 472-9690.

Highlights

In this study, the role that a nuclear localization signal in a regulatory protein (bICP0) encoded by bovine herpesvirus 1 plays in regulating beta-interferon activity was examined. These studies suggested that cytoplasmic localized bICP0 reduced beta-interferon promoter activity in low passage bovine cells. In addition, shuttling of bICP0 to the cytoplasm during productive infection required expression of a late viral gene product.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Infection of cattle with bovine herpesvirus 1 (BHV-1) leads to upper respiratory tract disorders, conjunctivitis, genital infections, encephalitis, abortions, multi-systemic fatal diseases in neonates and bovine respiratory disease, a life-threatening pneumonia (Jones and Chowdhury, 2007; Tikoo et al., 1995). BHV-1 has developed different mechanisms to suppress immune response during the course of infection. For example, CD8+ T cell recognition of infected cells is impaired (Hariharan et al., 1993; Hinkley et al., 1998; Nataraj et al., 1997). Furthermore, CD4+ T cell function is also impaired during acute infection of calves because BHV-1 infects CD4+ T cells and induces high levels of apoptosis (Winkler et al., 1999). The immune-suppressive properties of BHV-1 can lead to secondary infections, including life-threatening bacterial pneumonia (Carter et al., 1989; Griebel et al., 1990; Griebel et al., 1987a; Griebel et al., 1987b).

BHV-1 infection also inhibits several aspects of the innate immune response, reviewed in (Jones and Chowdhury, 2009). For example, a recent study demonstrated that BHV-1 gene expression represses bovine IFN- β response in bovine primary cells (da Silva and Jones, 2011). The BHV-1 infected cell protein 0 (bICP0) reduces human beta interferon (IFN- β) promoter activity (Henderson et al., 2005; Saira et al., 2007; Saira et al., 2009) by inducing proteasome dependent IRF3 (interferon regulatory factor 3) degradation (Saira et al., 2007) and interacting with IRF7 (Saira et al., 2009). IRF3 and IRF7 are cellular transcription factors that *trans*-activate the IFN- β promoter (Barnes et al., 2002; Doyle et al., 2002; Honda et al., 2005; Servant et al., 2002).

The bICP0 protein is constitutively expressed during productive infection because the bICP0 gene has an immediate early and early promoter, and both promoters are activated by bICP0 (Everett, 2000; Fraefel et al., 1994; Wirth et al., 1992). Alpha-herpesvirinae sub-family members encode an ICP0 homologue that stimulates productive infection, and these respective ICP0 homologues contain a well-conserved C₃HC₄ zinc RING finger near their N terminus, (Bowles et al., 1997; Bowles et al., 2000; Everett et al., 1995; Everett, 1988; Everett, 2000; Everett et al., 1993; Inman et al., 2001; Lium and Silverstein, 1997). ICP0 (Everett et al., 1999a; Everett et al., 1999b; Everett et al., 1997; Maul and Everett, 1994; Maul et al., 1993) and bICP0 (Gaudreault and Jones, 2011; Inman et al., 2001; Parkinson and Everett, 2000) localize with and disrupt promyelocytic leukemia (PML) protein-containing nuclear domains. The C₃HC₄ zinc RING finger domain located in bICP0 (Dia et al., 2005) and ICP0 (Boutell and Everett, 2003; Boutell et al., 2002; Van Sant et al., 2001) possess intrinsic E3 ubiquitin ligase activity, and induce ubiquitin dependent proteolysis of specific proteins (Everett et al., 1999a; Everett et al., 1997; Lees-Miller et al., 1996; Parkinson et al., 1999). Both bICP0 (Zhang and Jones, 2005) and ICP0 (Everett, 1988) contain a nuclear localization signal located near their C-terminus.

In contrast to humans or mice, cattle contain three different IFN- β genes that are regulated by distinct promoters (Valarcher et al., 2003; Wilson et al., 1983). We recently cloned and characterized the three bovine IFN- β promoters in a CAT reporter plasmid (da Silva and Jones, 2011). The bovine IFN- β 1 and IFN- β 3 promoters resemble the human IFN- β promoter more closely than IFN- β 2, and both are strongly activated by double stranded RNA (polyI:C) and IRF7. Sendai virus infection of low passage bovine cells or established bovine kidney cells increased IFN- β 1 levels (da Silva and Jones, 2011). Conversely, BHV-1 infection of low passage bovine cells did not increase IFN- β RNA levels unless *de novo* protein synthesis was inhibited (da Silva and Jones, 2011). In low passage bovine cells, IFN- β 1 RNA expression was induced when protein synthesis was blocked: whereas infection of established bovine kidney cells with BHV-1 increased IFN- β 3 RNA levels. These studies suggested that BHV-1 induced an IFN- β response during the initial stages of viral infection,

but viral protein synthesis subsequently blocked IFN- β signaling pathways in low passage bovine cells.

In this study, evidence is presented demonstrating that a bICP0 mutant that does not localize to the nucleus inhibits IFN- β promoter activity. For these studies, we used two low passage bovine cell lines obtained from kidney (BK) or testicles (BTest) from normal healthy calves. We felt this was important because BHV-1 blocks IFN- β RNA expression after infection of low passage bovine cells, but not established bovine cells (da Silva and Jones, 2011). Cytoplasmic localized bICP0 also induced degradation of IRF3. Cytoplasmic localized bICP0 is readily detected in low passage bovine cells, but not in established bovine cells, during productive infection (Gaudreault and Jones, 2011). Collectively, these studies suggest that a correlation exists between blocking IFN- β responses and localization of bICP0 to the cytoplasm.

METHODS

Cells

Low passage bovine kidney (BK) cells or low passage bovine testicle cells (BTest) were cultured in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (10 U/ml), and streptomycin (100 μ g/ml) in a humidified 5% CO₂ atmosphere at 37°C. Low passage cells were prepared from healthy calves using standard procedures.

Viruses

The Cooper strain of BHV-1 (wt virus) was obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Services, Ames, IA. BHV-1 stocks were prepared in bovine cells (CRIB).

Plasmids

The bovine IFN- β promoters were described previously (da Silva and Jones, 2011). The human IRF-3 and IRF-7 expression constructs were obtained from Luwen Zhang (University of Nebraska, Lincoln, NE). The bICP0 constructs were described in previous studies (Inman et al., 2001; Zhang et al., 2005).

Transient transfection assays

BK or BTest cells were transfected with the designated plasmids using Lipofectamine 2000 (Invitrogen, San Diego, CA) according to the manufacturer's instructions. Forty hours after transfection, cells were lysed by three freeze-thaw cycles in 250 mM Tris-HCL (pH7.4). CAT assays were performed with 0.2 μ Ci (7.4 KBq) [¹⁴C]-chloramphenicol (Amersham Biosciences, catalog no. CFA754) and 0.5 mM acetyl coenzyme A (Sigma, catalog no. A2181). Chloramphenicol and its acetylated forms were separated by thin-layer chromatography and CAT activity measured with a PhosphorImager (Molecular Dynamics, CA). CAT activity was expressed as the fold of induction relative to the vector control. Transfection experiments for CAT assays were repeated at least three times to confirm the results.

Immunofluorescence

Cells were plated in 60 mm culture dishes containing a sterilized cover slip at 16 hours prior to infection or transfection. Cells were washed twice with plain MEM and fixed with 4% para-formaldehyde for 10 minutes followed by three washes with PBS. Cells were permeabilized by incubating with 100% ethanol (−20°C) for 5 minutes. Slides were then washed three times and blocked in 3% BSA in PBS for 1 hour. The designated primary

antibody was incubated for 2 hours at room temperature. After three washes with 0.05% Tween 20 in PBS, slides were incubated with the secondary antibody for 1 hour in the dark. Secondary antibodies used were Alexa Fluor 488 goat anti-rabbit (A21050), Alexa Fluor 633 goat anti-mouse (A11008), and Alexa Fluor 647 donkey anti-rabbit (A31573) from Invitrogen. Images were obtained with a Bio-Rad confocal laser-scanning microscope (MRC-1024ES) with excitation/emission at 488/520 nm. Images are representative of three or more independent experiments.

RESULTS

bICP0 reduces bovine IFN- β promoter activity in transient transfection assays

Previous studies demonstrated that bICP0 inhibited human IFN- β promoter activity (Henderson et al., 2005; Saira et al., 2007; Saira et al., 2009). Since the bovine and human IFN- β promoters contain nucleotide differences in the four positive regulatory domains that are important for activation (da Silva, 2011), it was of interest to test whether bICP0 had an effect on the bovine IFN- β promoters. The bovine IFN- β 1 and IFN- β 3 promoters were used for these studies because they are active in transient transfection assays, in part because they only have four or two nucleotide differences respectively when compared to the human IFN- β promoter (da Silva and Jones, 2011). The IFN- β 2 promoter contains 11 mutations in the four positive regulatory domains when compared to the human IFN- β promoter, and does not respond to stimuli that activate other IFN- β promoters (da Silva and Jones, 2011). Consequently, the IFN- β 2 promoter was not used for these studies. Low-passage bovine kidney (BK) and bovine testicle (BTest) cells were used for these studies because when low passage bovine cells are infected with BHV-1 IFN- β expression is not readily detected unless *de novo* protein synthesis is blocked by treating cells with cycloheximide prior to infection (da Silva and Jones, 2011). Furthermore, BHV-1 productive infection of established bovine cells strongly increased IFN- β 3 RNA levels: whereas IFN- β 1 is primarily induced in low passage bovine cells following infection in the presence of cycloheximide (da Silva and Jones, 2011).

The transcription factors IRF7 and IRF3 were used for stimulating the respective activities of IFN- β 1 and IFN- β 3 because they efficiently stimulate these promoters in transient transfection assays (da Silva and Jones, 2011). IRF7 strongly activated the IFN- β 1 promoter as previously reported (da Silva and Jones, 2011), and bICP0 reduced promoter activity in a dose-dependent manner in BK and BTest cells (Figure 1A). IRF3 only trans-activates the bovine IFN- β 3 promoter (da Silva and Jones, 2011), and thus we examined the effect that bICP0 has on IRF3 mediated activation of the IFN- β 3 promoter (Figure 1B). In BK or BTest cells, bICP0 reduced IFN- β 3 promoter activity in a dose dependent manner. These studies provided evidence that bICP0 inhibited the bovine IFN- β 1 and IFN- β 3 promoters in low passage bovine cells, which were consistent with previous studies using the human IFN- β promoter (Henderson et al., 2005; Saira et al., 2007; Saira et al., 2009).

A bICP0 mutant lacking nuclear localization signals interferes with bovine IFN- β promoter activity

Additional studies were performed to test whether the nuclear localization signals in bICP0 were necessary for inhibiting bovine IFN- β promoter activity in low passage bovine cells (see Figure 2A for schematic of the bICP0 constructs used in this study). The rationale for these studies is bICP0 translocates between the nucleus and cytoplasm when low passage bovine cells are infected (Gaudreault and Jones, 2011), and IRF3 is localized to the cytoplasm prior to activation by viruses (Doyle et al., 2002; Fitzgerald et al., 2003). Furthermore, cytoplasmic localized HSV-1 encoded ICP0 inhibits IRF3 phosphorylation, dimerization, and nuclear translocation (Paladino et al., 2010). In low passage BK or BTest

cells, the Δ NcoI mutant, which lacks the nuclear localization signals of bICP0, reduced IFN- β 1 (Figure 2B) or IFN- β 3 (Figure 2C) promoter activity with the same efficiency as wt bICP0. This was unexpected because we believed that the functions of bICP0 were performed in the nucleus.

In both low passage cell types (Figure 2B and C), the 13G/51A mutant reduced IFN- β 1 and IFN- β 3 promoter activity as efficiently as wt bICP0. A previous study concluded that the 13G/51A mutant did not inhibit human IFN- β promoter activity as efficiently as wt bICP0 in 293 cells or an established bovine cell line (Henderson et al., 2005). These studies suggested that the zinc RING finger was not necessary for inhibiting IFN- β promoter activity in low passage bovine cell types or was not necessary for inhibiting the two bovine IFN- β promoters.

Cytoplasmic localized bICP0 induces degradation of IRF3

Although cytoplasmic bICP0 and HSV-1 ICP0 are capable of interfering with IRF3 dependent activation of IFN- β promoter activity, the two proteins inactivate IRF3 by distinct mechanisms (Paladino et al., 2010; Saira et al., 2007). With respect to bICP0, it seems clear that it reduces the steady state levels of IRF3 in transfected cells and an intact zinc RING finger is important for inducing IRF3 degradation (Saira et al., 2007). Consequently, we examined whether cytoplasmic localized bICP0 induced IRF3 degradation.

To test whether the Δ NcoI mutant protein induced IRF3 degradation, BTest cells were cotransfected with the Δ NcoI construct, wt bICP0, or an empty expression vector (pcDNA3.1) and a plasmid expressing IRF3. Twenty-four hours after transfection, IRF3 levels were reduced in BTest cells that were transfected with the Δ NcoI construct (Figure 3). The Δ NcoI mutant has consistently reduced IRF3 levels as efficiently as wt bICP0. When BTest cells were treated with a proteasome inhibitor (lactacystin) after transfection, IRF3 protein levels were higher suggesting that a functional proteasome was necessary for bICP0-induced degradation of IRF3.

Analysis of bICP0 localization in infected low passage bovine cells

Previous studies demonstrated the Δ NcoI mutant lacks the bICP0 nuclear localization signal, and consequently has a cytoplasmic localization in established or transformed cells (Zhang and Jones, 2005). However, the Δ NcoI mutant was not examined in low passage bovine cells. Consequently, confocal microscopy was performed using a wt bICP0 construct and the Δ NcoI mutant construct. Each of these constructs expresses a bICP0 protein that has a Flag-tag at the N-terminus (Zhang and Jones, 2005). For these studies, we used BTest cells because the efficiency of transfection was higher than in BK cells. The Δ NcoI mutant was localized to the cytoplasm in 100% of transfected BTest cells (more than 100 Δ NcoI bICP0 positive cells were counted; Figure 4A). After examining confocal images of many BTest cells expressing the Δ NcoI mutant, we concluded that the mutant bICP0 protein was not readily detected in the nucleus of transfected cells. Most of the cells transfected with the Δ NcoI mutant also contained speckles of Flag-staining in BTest (Figure 4A) or BK cells (data not shown) suggesting that a subset of cytoplasmic bICP0 was localized to specific structures located in the cytoplasm. As expected, wt bICP0 was localized in the nucleus of transfected cells, and mock-transfected cells were not stained with the Flag-specific antibody.

Treatment of low passage bovine cells with phosphonoacetic acid (PAA), an inhibitor of viral DNA replication, before and after BHV-1 infection increased the levels of IFN- β RNA compared to cultures not treated with PAA (da Silva and Jones, 2011). However, PAA did not restore IFN- β 1 RNA levels after infection with BHV-1 to the same extent as inhibiting

de novo protein synthesis by treating cells with cycloheximide (da Silva and Jones, 2011). Studies with HSV-1 demonstrated that PAA treatment inhibits expression of viral late proteins and consequently ICP0 translocation from the nucleus to the cytoplasm is not readily detected (Lopez et al., 2001). Furthermore, ICP0 nuclear retention prevented HSV-1 from inactivating IRF3 (Paladino et al., 2010). Therefore we tested whether PAA treatment interfered with bICP0 translocation to the cytoplasm. In agreement with previous studies (Gaudreault and Jones, 2011) increasing levels of bICP0 were detected in the cytoplasm of infected BTest cells as a function of time after infection. At 16 hours after infection, cytoplasmic bICP0 was readily detected in most infected BTest cells (Figure 3B). However, bICP0 was not readily detected in the cytoplasm of BTest cells at 16 hours after infection when cultures were treated with PAA. These observations suggested that a late viral encoded or induced factor was necessary for cytoplasmic localization of bICP0 and that bICP0 translocation to the cytoplasm correlated with IFN- β RNA repression.

DISCUSSION

BHV-1 infection completely blocked induction of the three subtypes of IFN- β RNA in low passage BK cells (da Silva and Jones, 2011), BTest cells, or low passage bovine turbinate cells (data not shown). In contrast, BHV-1 infection of established bovine kidney cells led to increased IFN- β 3 RNA levels and to a lesser extent IFN- β 1 and IFN- β 2 RNA levels (da Silva and Jones, 2011; Perez et al., 2008). In established bovine kidney or rabbit skin cells, cytoplasmic bICP0 was not readily detected after infection (Gaudreault and Jones, 2011). This study (Figure 4) and a previous study (Gaudreault and Jones, 2011) demonstrated that bICP0 was readily detected in the cytoplasm after infection of low passage bovine cells. HSV-1 encoded ICP0 is also detected in the cytoplasm of low-passage or established cells (Kalamvoki and Roizman, 2010; Kawaguchi et al., 1997; Lopez et al., 2001). Collectively, these observations indicated there is a correlation between blocking induction of IFN- β RNA expression after infection and cytoplasmic localization of bICP0.

This study provided evidence that when bICP0 was localized to the cytoplasm it inhibited IFN- β promoter activity and induced IRF3 degradation in low passage bovine cells. Cytoplasmic localized bICP0 also reduces the levels of the promyelocytic leukemia (PML) protein (Fiorito et al., 2009). Non-nuclear PML is enriched at the endoplasmic reticulum and mitochondria-associated membranes (Giorgi et al., 2010). PML is implicated in regulating innate immune responses (Everett and Chelbi-Alix, 2007; Ullman and Hearing, 2008) suggesting cytoplasmic localized bICP0 also influences innate immune responses by degrading PML. The ability of cytoplasmic bICP0 to induce degradation of IRF3, an important anti-viral cellular protein that is localized in the cytoplasm unless activated, is logical because it would prevent IRF3 from entering the nucleus and activating IFN- β promoter activity. Since wt bICP0 was primarily detected in the nucleus of transfected cells, we suggest that when IRF3 is activated and enters the nucleus; nuclear-localized bICP0 can also induce IRF3 degradation. Conversely, a subset of wt bICP0 may shuttle from the nucleus to the cytoplasm and promote IRF3 degradation. In summary, these studies suggested that cytoplasmic localized bICP0 plays an important role in interfering with innate immune responses.

In previous studies, we examined the effects of bICP0 on the human IFN- β promoter in established or transformed non-bovine cell types (Henderson et al., 2005; Saira et al., 2007). In 293 cells, the Δ NcoI mutant did not effectively inhibit the human IFN- β promoter (Saira et al., 2007). However, a more extensive bICP0 mutant (Δ bICP0) that only localizes to the cytoplasm of transfected mouse neuroblastoma cells inhibited the human IFN- β promoter with similar efficiency as wt bICP0 (Henderson et al., 2005). We believe these differences

may be due to cell type specific events that are induced by bICP0 and these deletion mutants.

The importance of the bICP0 zinc RING finger, in the context of inhibiting IFN- β promoter activity, is dependent on the cell type and how the human IFN- β promoter is stimulated. For example, the zinc RING finger is important for inhibiting IFN- β promoter activity in human 293 cells or mouse neuroblastoma cells when the human IFN- β promoter is activated by IRF7 or protein kinases (TBKI or IKK- ϵ) that stimulate the IFN- β signaling pathway (Henderson et al., 2005). Furthermore, the zinc RING finger is important for inhibiting the human IFN- β promoter in established bovine testicle cells, but not 293 or neuro-2A cells, when the promoter is activated by IRF3 (Henderson et al., 2005). Additional domains in the C-terminal one-half of the protein are important for inhibiting the human IFN- β promoter (Saira et al., 2007) suggesting these domains may overcome the two point mutations in the 13G/51A mutant in certain cell types. A mutant virus containing the 51A mutation has reduced virus shedding during acute infection and does not reactivate from latency indicating a wt zinc RING finger is necessary for virulence and pathogenesis (Saira et al., 2008). Since BHV-1 only infects cattle, rabbits, and other ungulates, it seems wise to examine these complicated virus host interactions of BHV-1 in low passage bovine cells. These findings further suggest that bICP0 interacts with more than one cellular regulatory protein, and these interactions may occur in a cell type specific fashion.

Acknowledgments

This research was supported by grants from the USDA and Agriculture and Food Research Initiative Competitive Grants Program (08-00891 and 09-01653). A grant to the Nebraska Center for Virology (1P20RR15635), in particular the confocal microscopy core facility, also supported certain aspects of these studies. Natasha Gaudreault was partially supported by a fellowship from a Ruth L. Kirschstein National Research Service Award 1 T32 AIO60547 (National Institute of Allergy and Infectious Diseases).

References

- Barnes B, Lubyova B, Pitha PM. On the control of IRF in host defense. *J Interferon Cytokine Res.* 2002; 22:59–71. [PubMed: 11846976]
- Boutell C, Everett RD. The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with an ubiquitinates p53. *J Biol Chem.* 2003; 278(38):36596–36602. [PubMed: 12855695]
- Boutell C, Sadis S, Everett RD. Herpes simplex virus type 1 immediate-early protein ICP0 and its isolated RING finger domain act as ubiquitin E3 ligases in vitro. *J Virol.* 2002; 76:841–850. [PubMed: 11752173]
- Bowles DE V, Holden R, Zhao Y, O'Callaghan DJ. The ICP0 protein of equine herpesvirus 1 is an early protein that independently transactivates expression of all classes of viral promoters. *J Virol.* 1997; 71(7):4904–14. [PubMed: 9188552]
- Bowles DE, Kim SK, O'Callaghan DJ. Characterization of the trans-activation properties of equine herpesvirus 1 EICP0 protein. *J Virol.* 2000; 74(3):1200–8. [PubMed: 10627530]
- Carter JJ, Weinberg AD, Pollard A, Reeves R, Magnuson JA, Magnuson NS. Inhibition of T-lymphocyte mitogenic responses and effects on cell functions by bovine herpesvirus 1. *J Virol.* 1989; 63(4):1525–30. [PubMed: 2538643]
- da Silva LF, Jones C. Infection of cultured bovine cells with bovine herpesvirus 1 (BHV-1) or Sendai virus induces different beta interferon subtypes. *Virus Res.* 2011; 157:54–60. [PubMed: 21316405]
- Dia L, Zhang B, Fan J, Gao X, Sun S, Yang K, Xin D, Jin N, Geng Y, Wang C. Herpes virus proteins ICP0 and BICP0 can activate NF- κ B by catalyzing I κ Ba ubiquitination. *Cellular Signalling.* 2005; 17:217–229. [PubMed: 15494213]
- Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T, Rao G, Sun R, Haberland M, Modlin R, Cheng G. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. *Immunity.* 2002; 17:251–263. [PubMed: 12354379]

- Everett R, O'Hare P, O'Rourke D, Barlow P, Orr A. Point mutations in the herpes simplex virus type 1 Vmw110 RING finger helix affect activation of gene expression, viral growth, and interaction with PML-containing nuclear structures. *J Virol.* 1995; 69(11):7339–44. [PubMed: 7474166]
- Everett RD. Analysis of the functional domains of herpes simplex virus type 1 immediate-early polypeptide Vmw110. *J Mol Biol.* 1988; 202(1):87–96. [PubMed: 2845096]
- Everett RD. ICP0, a regulator of herpes simplex virus during lytic and latent infection. *Bioessays.* 2000; 22(8):761–70. [PubMed: 10918307]
- Everett RD, Barlow P, Milner A, Luisi B, Orr A, Hope G, Lyon D. A novel arrangement of zinc-binding residues and secondary structure in the C3HC4 motif of an alpha herpes virus protein family. *J Mol Biol.* 1993; 234(4):1038–47. [PubMed: 8263911]
- Everett RD, Earnshaw WC, Findlay J, Lomonte P. Specific destruction of kinetochore protein CENP-C and disruption of cell division by herpes simplex virus immediate-early protein Vmw110. *EMBO J.* 1999a; 18(6):1526–38. [PubMed: 10075924]
- Everett RD, Lomonte P, Sternsdorf T, van Driel R, Orr A. Cell cycle regulation of PML modification and ND10 composition. *J Cell Sci.* 1999b; 112(Pt 24):4581–8. [PubMed: 10574707]
- Everett RD, Meredith M, Orr A, Cross A, Kathoria M, Parkinson J. A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein [corrected and republished article originally printed in *EMBO J* 1997 Feb 3;16(3):566–77]. *EMBO J.* 1997; 16(7):1519–30. [PubMed: 9130697]
- Everett RD, Chelbi-Alix MK. PML and PML nuclear bodies: implications in antiviral defence. *Biochimie.* 2007; 74:819–830.
- Fiorito F, Marfe G, Granato GE, Ciarcia R, De Blasio E, Tafani M, Florio S, De Martino L, Muzi G, Pagnini U, Giordano A. 2,3,7,8-Tetrachlorodibenzo-p-dioxin modifies expression and nuclear/cytosolic localization of bovine herpesvirus 1 immediate-early protein (bICP0) during infection. *J Cell Biochem.* 2009; 111(2):333–42. [PubMed: 20506271]
- Fitzgerald KA, McWhirter SM, Faja KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T. IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nature.* 2003; 4:491–496.
- Fraefel C, Zeng J, Choffat Y, Engels M, Schwyzer M, Ackermann M. Identification and zinc dependence of the bovine herpesvirus 1 transactivator protein BICP0. *J Virol.* 1994; 68(5):3154–62. [PubMed: 8151780]
- Gaudreault N, Jones C. Regulation of promyelocytic leukemia (PML) protein levels and cell morphology by bovine herpesvirus 1 infected cell protein 0 (bICP0) and mutant bICP0 proteins that do not localize to the nucleus. *Virus Res.* 2011; 156:17–24. [PubMed: 21215282]
- Giorgi C, Ito K, Lin HK, Santangelo C, Wieckowski MR, Lebiedzinska M, Bononi A, Bonora M, Duszynski J, Bernardi R, Rizzuto R, Tacchetti C, Pinton P, Pandolfi PP. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science.* 2010; 330:1247–1251. [PubMed: 21030605]
- Griebel P, Ohmann HB, Lawman MJ, Babiuk LA. The interaction between bovine herpesvirus type 1 and activated bovine T lymphocytes. *J Gen Virol.* 1990; 71(Pt 2):369–77. [PubMed: 2155290]
- Griebel P, Qualtiere L, Davis WC, Gee A, Bielefeldt Ohmann H, Lawman MJ, Babiuk LA. T lymphocyte population dynamics and function following a primary bovine herpesvirus type-1 infection. *Viral Immunol.* 1987a; 1(4):287–304. [PubMed: 3509949]
- Griebel PJ, Qualtiere L, Davis WC, Lawman MJ, Babiuk LA. Bovine peripheral blood leukocyte subpopulation dynamics following a primary bovine herpesvirus-1 infection. *Viral Immunol.* 1987b; 1(4):267–86. [PubMed: 3509948]
- Hariharan MJ, Nataraj C, Srikumaran S. Down regulation of murine MHC class I expression by bovine herpesvirus 1. *Viral Immunol.* 1993; 6(4):273–84. [PubMed: 8166934]
- Henderson G, Zhang Y, Jones C. The bovine herpesvirus 1 gene encoding infected cell protein 0 (bICP0) can inhibit interferon-dependent transcription in the absence of other viral genes. *J Gen Virol.* 2005; 86:2697–2702. [PubMed: 16186222]
- Hinkley S, Hill AB, Srikumaran S. Bovine herpesvirus-1 infection affects the peptide transport activity in bovine cells. *Virus Res.* 1998; 53(1):91–6. [PubMed: 9617772]

- Honda K, Yanai H, Negishi H, Asagiri M, Saton M, Mizutani T, Shimada N, Ohba Y, Takaoka A, Yoshida N, Taniguchi T. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature*. 2005; 434:772–777. [PubMed: 15800576]
- Inman M, Zhang Y, Geiser V, Jones C. The zinc ring finger in the bICP0 protein encoded by bovine herpes virus-1 mediates toxicity and activates productive infection. *J Gen Virol*. 2001; 82:483–492. [PubMed: 11172088]
- Jones C. Regulation of innate immune responses by bovine herpesvirus 1 and infected cell protein 0. *Viruses*. 2009; 1:255–275. [PubMed: 21994549]
- Jones C, Chowdhury S. 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. *Anim Health Res Rev*. 2007; 8(2):187–205. [PubMed: 18218160]
- Kalamvoki M, Roizman B. Role of the herpes simplex virus ICP0 in the transactivation of genes introduced by infection or transfection: a reappraisal. *J Virol*. 2010; 84:4222–4228. [PubMed: 20164233]
- Kawaguchi Y, Bruni R, Roizman B. Interaction of herpes simplex virus 1 alpha regulatory protein ICP0 with elongation factor 1delta: ICP0 affects translational machinery. *J Virol*. 1997; 71(2):1019–24. [PubMed: 8995621]
- Lees-Miller SP, Long MC, Kilvert MA, Lam V, Rice SA, Spencer CA. Attenuation of DNA-dependent protein kinase activity and its catalytic subunit by the herpes simplex virus type 1 transactivator ICP0. *J Virol*. 1996; 70(11):7471–7. [PubMed: 8892865]
- Lium EK, Silverstein S. Mutational analysis of the herpes simplex virus type 1 ICP0 C3HC4 zinc ring finger reveals a requirement for ICP0 in the expression of the essential alpha27 gene. *J Virol*. 1997; 71(11):8602–14. [PubMed: 9343218]
- Lopez P, Van Sant C, Roizman B. Requirements for the nuclear-cytoplasmic translocation of infected-cell protein 0 of herpes simplex virus type 1. *J Virol*. 2001; 75:3832–3840. [PubMed: 11264372]
- Maul GG, Everett RD. The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICP0. *J Gen Virol*. 1994; 75(Pt 6):1223–1233. [PubMed: 8207389]
- Maul GG, Guldner HH, Spivack JG. Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *J Gen Virol*. 1993; 74(Pt 12):2679–90. [PubMed: 8277273]
- Nataraj C, Eidmann S, Hariharan MJ, Sur JH, Perry GA, Srikumaran S. Bovine herpesvirus 1 downregulates the expression of bovine MHC class I molecules. *Viral Immunol*. 1997; 10(1):21–34. [PubMed: 9095529]
- Paladino P, Collins SE, Mossman KL. Cellular localization of the herpes simplex virus ICP0 protein dictates its ability to block IRF-3 mediated innate immune responses. *PLOS one*. 2010; 5:e10428. [PubMed: 20454685]
- Parkinson J, Everett RD. Alpha herpesvirus proteins related to herpes simplex virus type 1 ICP0 affect cellular structures and proteins. *J Virol*. 2000; 74(21):10006–10017. [PubMed: 11024129]
- Parkinson J, Lees-Miller SP, Everett RD. Herpes simplex virus type 1 immediate-early protein vmw110 induces the proteasome-dependent degradation of the catalytic subunit of DNA-dependent protein kinase. *J Virol*. 1999; 73(1):650–7. [PubMed: 9847370]
- Perez S, Meyer F, Saira K, Doster A, Jones C. Premature expression of the latency-related RNA encoded by bovine herpesvirus 1 correlates with higher levels of beta interferon RNA expression in productively infected cells. *J Gen Virol*. 2008; 89:1338–1345. [PubMed: 18474548]
- Saira, K. PhD. University of Nebraska; 2008. Functional analysis of the bovine herpesvirus-1 gene encoding bICP0, a promiscuous trans-activator, that stimulates productive infection and interferon signaling pathways.
- Saira K, Zhou Y, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) induces degradation of interferon response factor 3 (IRF3), and consequently inhibits beta interferon promoter activity. *J Virol*. 2007; 81:3077–3086. [PubMed: 17215277]
- Saira K, Zhou Y, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) induces degradation of interferon response factor 3 and, consequently, inhibits beta interferon promoter activity. *J Virol*. 2007; 81(7):3077–3086. [PubMed: 17215277]

- Saira K, Zhou Y, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) associates with interferon regulatory factor 7 and consequently inhibits beta interferon promoter activity. *J Virol.* 2009; 83(8):3977–3981. [PubMed: 19176627]
- Saira K, Jones C. The infected cell protein 0 encoded by bovine herpesvirus 1 (bICP0) associates with interferon regulatory factor 7 (IRF7), and consequently inhibits beta interferon promoter activity. *J Virol.* 2009; 83:3977–3981. [PubMed: 19176627]
- Servant MJ, Tenover B, Lin R. Overlapping and distinct mechanisms regulating IRF-3 and IRF-7 function. *J of Interferon and Cytokine Research.* 2002; 22:49–58. [PubMed: 11846975]
- Tikoo SK, Campos M, Babiuk LA. Bovine herpesvirus 1 (BHV-1): biology, pathogenesis, and control. *Adv Virus Res.* 1995; 45:191–223. [PubMed: 7793325]
- Ullman AJ, Hearing P. Cellular proteins PML and Daxx mediate an innate antiviral defense antagonized by the adenovirus E4 ORF3 protein. *J Virol.* 2008; 82:7325–7335. [PubMed: 18480450]
- Valarcher JF, Furze J, Wyld S, Cook R, Conzelman KK, Taylor G. Role of alpha/beta interferons in the attenuation and immunogenicity of recombinant bovine respiratory syncytial viruses lacking NS proteins. *J Virol.* 2003; 77:8426–8439. [PubMed: 12857912]
- Van Sant C, Hagglund R, Lopez P, Roizman B. The infected cell protein 0 of herpes simplex virus 1 dynamically interacts with proteasomes, binds and activates the cdc34 E2 ubiquitin- conjugating enzyme, and possesses in vitro E3 ubiquitin ligase activity. *Proc Natl Acad Sci U S A.* 2001; 98(15):8815–20. [PubMed: 11447293]
- Wilson V, Jeffreys AJ, Barrie PA. A comparison of vertebrate interferon gene families detected by hybridization with human interferon DNA. *J Mol Biol.* 1983; 166:457–475. [PubMed: 6306247]
- Winkler MT, Doster A, Jones C. Bovine herpesvirus 1 can infect CD4(+) T lymphocytes and induce programmed cell death during acute infection of cattle. *J Virol.* 1999; 73(10):8657–68. [PubMed: 10482619]
- Wirth UV, Fraefel C, Vogt B, Vlcek C, Paces V, Schwyzer M. 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.* 1992; 66(5):2763–72. [PubMed: 1313901]
- Zhang Y, Jones C. Identification of functional domains within the bICP0 protein encoded by BHV-1. *J Gen Virol.* 2005; 86:879–886. [PubMed: 15784882]
- Zhang Y, Zhou J, Jones C. Identification of functional domains within the bICP0 protein encoded by bovine herpesvirus 1. *J Gen Virol.* 2005; 86(Pt 4):879–86. [PubMed: 15784882]

Figure 1. Repression of bovine IFN-β1 and IFN-β3 promoter activities by bICP0

Confluent monolayers of bovine testicles cells (BTest) and BK cells were cotransfected with 1 μg of a CAT reporter plasmid containing the bovine IFN-β1 (Panel A) or IFN-β3 promoter (Panel B), 1 μg of IRF7, or 1 μg of IRF3, and increasing levels of a bICP0 expression construct (1, 2, or 3 μg) that was previously described (Inman et al., 2001). Plasmid DNA was maintained at the same concentration by inclusion of an empty expression vector (pcDNA3.1). Approximately 40 hours after transfection, CAT expression was measured as previously described (da Silva and Jones, 2011). Activation of the designated IFN-β promoter in the presence of IRF3 or IRF7 was arbitrarily set at 100%. The results are the mean of at least 3 independent experiments.

Figure 2. Cytoplasmic bICP0 represses bovine IFN-β promoter activity

Panel A: Schematic of wt bICP0, the 13G/51A mutant, and a deletion mutant lacking DNA sequences that contain the nuclear localization signal (ΔNcoI). Cysteine to glycine and Cysteine to alanine mutations were inserted into the 13th and 51st amino acids of bICP0, which are included the zinc RING finger (Inman et al., 2001). The construction of the ΔNcoI mutant was previously described (Zhang et al., 2005) and contains a deletion from NcoI-XhoI (amino acids 607–676).

BTest cells (Panel B) or BK cells (Panel C) were cotransfected with 1 μg of a CAT reporter plasmid containing the IFN-β1 or IFN-β3 promoters, 1 μg of IRF3 or 1 μg of IRF7, and 1 μg of the designated bICP0 expression plasmid. Total plasmid DNA levels were maintained at the same concentration by inclusion of a blank expression vector (pcDNA3.1). Cells extracts were collected at approximately 40 hours after transfection and CAT expression measured as previously described (da Silva and Jones, 2011).

Figure 3. Cytoplasmic localized bICP0 reduces IRF3 protein levels in transiently transfected cells

BTest cells were transfected with 1 µg of the designed plasmids. At five hours after transfection, cells were treated with 15 µM lactacystin or dimethyl sulfoxide (DMSO), which was used to suspend lactacystin (Sigma Aldrich, catalog number L6785). Whole-cell lysate was collected at 24 hours after transfection. Western blot analysis performed with rabbit anti-IRF3 antibody (Santa Cruz, catalog number FL-425) and mouse anti-FLAG antibody (Sigma Aldrich, catalog number F-1804) for detecting IRF-3 and bICP0 protein respectively, as previously described (da Silva and Jones, 2011; Gaudreault and Jones, 2011).

Figure 4. Localization of bICP0 and the Δ NcoI mutant in low passage bovine cells

Panel A: BTest cells were transfected with the designated plasmids. Forty hours after transfection cells were processed for confocal microscopy as described in the methods section.

Panel B: Monolayers containing approximately 2×10^6 bovine turbinate (BT) cells were mock infected or infected with BHV-1 (moi = 2) for 16 hours in the presence or absence of 400 μ g/ml of phosphonoacetic acid (PAA; Sigma Aldrich catalogue #284270).

Cells were processed for confocal microscopy and incubated with rabbit anti-bICP0 antibody (green stain) as described previously (Gaudreault and Jones, 2011; Zhang et al., 2005). Nuclear DNA was stained with DAPI, blue, (4',6-diamidino-2-phenylindole). The results are representative of three or more experiments.