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REVIEW

Regulation of the latency–reactivation cycle by products encoded by the bovine herpesvirus 1 (BHV-1) latency-related gene

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Abstract Like other α-herpesvirinae subfamily members, the primary site for bovine herpesvirus 1 (BHV-1) latency is ganglionic sensory neurons. Periodically BHV-1 reactivates from latency, virus is shed, and consequently virus transmission occurs. Transcription from the latency-related (LR) gene is readily detected in neurons of trigeminal ganglia (TG) of calves or rabbits latently infected with BHV-1. Two micro-RNAs and a transcript encompassing a small open reading frame (ORF-E) located within the LR promoter can also be detected in TG of latently infected calves. A BHV-1 mutant that contains stop codons near the beginning of the first open reading frame (ORF2) within the major LR transcript (LR mutant virus) has been characterized. The LR mutant virus does not express ORF2, a reading frame that lacks an initiating ATG (reading frame B), and has reduced expression of ORF1 during productive infection. The LR mutant virus does not reactivate from latency following dexamethasone treatment suggesting that LR protein expression regulates the latency–reactivation cycle. Higher levels of apoptosis occur in TG neurons of calves infected with the LR mutant viruses when compared to wild-type BHV-1 indicating that the anti-apoptotic properties of the LR gene is necessary for the latency–reactivation cycle. ORF2 inhibits apoptosis and regulates certain viral promoters, in part, because it interacts with three cellular transcription factors (C/EBP-alpha, Notch1, and Notch3). Although ORF2 is important for the latency–reactivation cycle, we predict that other LR gene products play a supportive role during life-long latency in cattle.

Keywords Latency · Sensory neurons · Bovine herpesvirus 1 · Apoptosis

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Pathogenic potential of BHV-1

Bovine herpesvirus 1 (BHV-1), an α-herpesvirinae subfamily member, induces a variety of clinical signs in the upper respiratory tract and is immune suppressive. For example, BHV-1 infection inhibits cell-mediated immunity (Carter et al. 1989; Griebel et al. 1987a,b, 1990), CD8+ T-cell recognition of infected cells (Hariharan et al. 1993; Hinkley et al. 1998; Koppers-Lalic et al. 2005; Nataraj et al. 1997), and induces apoptosis in CD4+ T cells (Eskra and Splitter 1997; Winkler et al. 1999). A viral regulatory protein, bICP0, inhibits interferon-dependent transcription (Henderson et al. 2005; Jones 2009; Saira et al. 2007; Saira and Jones 2009). The immune-suppressive activities of BHV-1 can lead to bovine respiratory disease complex (BRDC) (Tikoo et al. 1995). In addition to BHV-1, additional RNA viruses can suppress bovine immune responses during productive infection, thus increasing the frequency of secondary bacterial infections and BRDC, reviewed by Collins et al. (2001) and Srikumaran et al. (2007).

Infection also erodes mucosal surfaces of the upper respiratory tract, which promotes establishment of bacterial pathogens, for example *Mannheimia (M.) haemolytica*, in the lower respiratory tract (Highlander et al. 2000; Highlander 2001; Zecchinon et al. 2005). BHV-1 productive infection increases neutrophil adhesion and activation (Rivera-Rivas et al. 2009), which may amplify the effects

of *M. haemolytica*, a gram-negative bacterium (Songer and Post 2005) that exists as normal flora within the upper respiratory tract of healthy ruminants (Frank 1984). This commensal relationship is disrupted following stress or viral co-infections (Rice et al. 2008), then *M. haemolytica* quickly becomes the predominant organism responsible for bronchopneumonia that is associated with BRDC (Highlander et al. 2000; Highlander 2001; Zecchinon et al. 2005).

The latency–reactivation cycle is crucial for survival of BHV-1 in nature

Infection of permissive cells (Devireddy and Jones 1999) or acute infection of calves (Winkler et al. 1999) with BHV-1 leads to rapid cell death, in part due to apoptosis. Viral gene expression is temporally regulated in three distinct phases: immediate early (IE), early (E), or late (L). IE gene expression is stimulated by a virion component, α-TIF (Misra et al. 1994, 1995). Two IE transcription units exist: IE transcription unit 1 (IEtu1) and IEtu2 (Fraefel et al. 1994; Wirth et al. 1991, 1992). IEtu1 encodes functional homologues of two HSV-1 IE proteins, ICP0 and ICP4. The HSV-1 ICP4 protein is a sequence-specific DNA binding protein that stimulates most E as well as L promoters, and is necessary for productive infection (Smith et al. 1993). IEtu2 encodes a protein that is similar to an important HSV IE protein, ICP22 (Wirth et al. 1991). The HSV-1 ICP22 protein is not required for productive infection of cultured cells, but promotes viral transcription by modifying the host RNA polymerase II (Rice et al. 1995). In general, IE proteins activate E gene expression, and viral DNA replication ensues. L gene expression is also activated by bICP0, culminating in virion assembly and release. bICP0 is crucial for productive infection because it activates all viral promoters, and bICP0 is expressed at high levels throughout productive infection, in part, because it has an IE and E promoter (Fraefel et al. 1994; Wirth et al. 1989, 1991, 1992).

Following acute infection, BHV-1 establishes latency in sensory neurons (Jones 1998, 2003, 2009). Periodically, BHV-1 reactivates from latency and sheds infectious virus, which is readily transmitted to uninfected cattle. As a result of the latency–reactivation cycle, BHV-1 is widespread in cattle. It is estimated that nearly all dairy cows are latently infected whereas fewer beef cattle are latently infected because they are slaughtered at a younger age (reviewed in Jones 1998, 2003). Ganglionic neurons are the main site of latency for BHV-1 and other α-herpesvirinae subfamily members. Viral particles enter the peripheral nervous system via cell–cell spread. If infection is initiated within the oral, nasal, or ocular cavity, the primary site for latency is sensory neurons in trigeminal ganglia (TG). Viral gene expression (Schang and Jones 1997) and infectious virus (Inman et al. 2002) are detected in TG from 2 to 6 days after infection. Viral gene expression is then extinguished, a significant number of infected neurons survive, and these surviving infected neurons harbor viral genomes (establishment of latency). Latent or persistent infections also appears to occur in non-neural sites. For example, BHV-1 DNA is consistently detected in tonsils (Winkler et al. 2000a,b), peripheral blood cells (Fuchs et al. 1999), lymph nodes, and spleen even when infectious virus is not detected (Mweene et al. 1996). Infectious virus and abundant lytic cycle viral gene expression is not readily detected during the maintenance of latency.

The stress induced by moving cattle from one location to another as well as weaning increases corticosteroid levels, which can trigger BHV-1 reactivation from latency. Administration of the synthetic corticosteroid dexamethasone (DEX) to latently infected calves or rabbits consistently initiates reactivation from latency (Inman et al. 2002; Jones 1998, 2003; Jones et al. 2000; Rock et al. 1992). Lytic cycle viral gene expression is detected in neurons of latently infected calves 6 h after DEX treatment (Winkler et al. 2000a,b, 2002). Finally, DEX treatment of latently infected calves induces apoptosis of T cells that persist in TG after infection (Winkler et al. 2002). Persistence of T cells in TG of humans or mice latently infected with HSV-1 also occurs (Cantin et al. 1995; Halford et al. 1996; Liu et al. 1996; Shimeld et al. 1995, 1996, 1997; Theil et al. 2003) and is proposed to play a role in maintaining latency (Khanna et al. 2003; Knickelbein et al. 2008; Liu et al. 2000, 2001; Prbhakaran et al. 2005).

Abundant expression of the latency-related gene occurs in TG of latently infected cattle

LR-RNA is abundantly transcribed in latently infected neurons (Kutish et al. 1990; Rock et al. 1987, 1992). During productive infection, the 5′ terminus of LR RNA is at nucleotide 724, but the start site of LR-mRNA in TG is further upstream (Bratanich et al. 1992; Hossain et al. 1995) (Fig. 1a). LR-RNA is transcribed antisense with respect to the IE and E gene transcript (IE2.9/E2.6) that encodes bICP0 (Fig. 1b). The LR gene has two open reading frames (ORF), ORF-1 and ORF-2, and two reading frames that lack an initiating ATG (RF-B and RF-C) (Fig. 1b). A peptide antibody directed against the N terminus of ORF-2 recognizes a protein encoded by the LR gene (Hossain et al. 1995; Jiang et al. 1998, 2004). A fraction of LR-RNA is polyadenylated and alternatively spliced in TG, suggesting these transcripts are translated into more than one LR protein (Devireddy and Jones 1999; Hossain et al. 1995). DEX represses LR promoter activity (Jones et al. 1990) and reduces LR-RNA levels (Rock et al. 1992) suggesting LR gene products do not directly influence reactivation from latency.

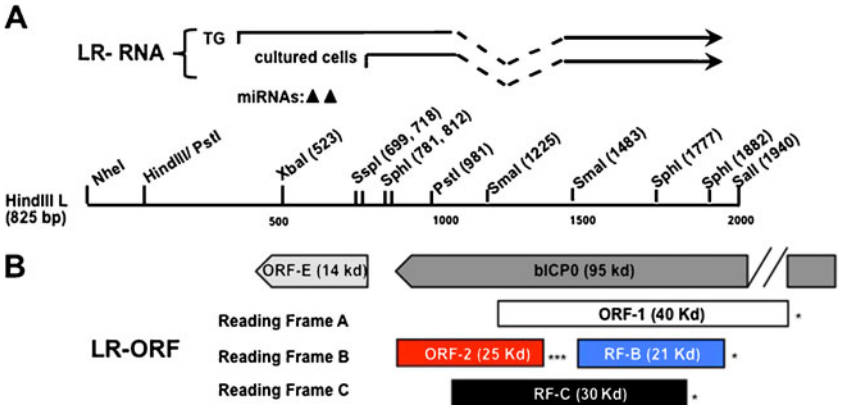

Fig. 1 Schematic of the LR gene and surrounding genes. **a** Partial restriction map of LR gene, location of LR-RNA, and position of two miRNAs located within the LR gene. The start sites for LR transcription during latency and productive infection were previously described (Devireddy and Jones 1998; Hossain et al. 1995). **b** Organization of LR ORFs and 3′ terminus of bICP0. ORF-1 and ORF-2 are located in the LR gene and have the potential to encode a 40- or 25-kDa protein, respectively. Reading frames B (*RF-B*) and C (*RF-C*) contain an open reading frame, but lacks an initiating Met. The *asterisks* (*) denote the position of stop codons that are in frame with the respective ORF. The positions of ORF-E and bICP0, which are antisense to LR-RNA, are also shown

A mutant BHV-1 strain that contains three stop codons near the beginning of ORF-2 was constructed to test whether LR protein expression regulates viral growth in cattle (Inman et al. 2001a,b). Antibodies directed against ORF-2 and RF-B recognize proteins expressed in bovine cells infected with wild-type (wt) or the LR rescued virus, but not when cells are infected with the LR mutant (Jiang et al. 2004). ORF-1 protein expression is reduced, but not blocked, following infection of cultured bovine cells with the LR mutant virus (Meyer et al. 2007a). ORF-1 can also be detected in a subset of TG neurons in calves latently infected with wt BHV-1, but not the LR mutant virus (Meyer et al. 2007a). Calves infected with the LR mutant virus exhibit diminished clinical symptoms and reduced shedding of infectious virus from the eye, TG, or tonsil when compared to calves infected with wt or the LR rescued virus (Inman et al. 2001a,b, 2002; Perez et al. 2005). Conversely, the LR mutant virus had similar growth properties in productively infected bovine kidney cells and the nasal cavity of calves during acute infection. LR-RNA is detected by RT–PCR in TG of calves infected with the LR mutant virus, but reduced levels of viral DNA are present in TG of latently infected calves (Inman et al. 2002). In spite of lower levels of infectious virus being detected in TG of calves infected with the LR mutant, higher levels of apoptosis occur in TG neurons during the late stages of acute infection (Lovato et al. 2003) indicating wt expression of LR gene products protect neurons from cell death during establishment and perhaps maintenance of latency. The LR mutant virus also induces higher levels of beta-interferon (IFN-β) RNA during productive infection of cultured bovine cells as well as in tonsils of acutely infected calves, in part because LR-RNA is prematurely expressed (Perez et al. 2005). This implies that premature expression of a specific LR gene product stimulates IFN-β expression or that the mutation prevented expression of a product that inhibits IFN-β RNA expression. It does not appear that ORF2 directly inhibits IFN-β promoter activity (unpublished data).

The LR mutant virus does not reactivate from latency following treatment with dexamethasone DEX (Inman et al. 2002). Conversely, all calves latently infected with wt virus or the LR rescued virus reactivated from latency after DEX treatment, as judged by shedding of infectious virus from the nasal or ocular cavity. Although the LR mutant virus does not successfully reactivate from latency, DEX treatment of calves latently infected with the LR mutant consistently induced expression of bICP0, but not bICP4 or the late transcript encoding glycoprotein C (Workman et al. 2009). Like the LR mutant virus, bICP4 transcription was not consistently detected during DEX-induced reactivation from latency in calves (Workman et al. 2009). However, wt BHV-1 consistently expresses the late glycoprotein C transcript, which correlated with reactivation from latency. Since bICP4 and bICP0 genes share a common immediate early promoter (Fig. 2b), this suggests the IEtu1 promoter is not consistently activated during DEX induced reactivation from latency.

The bICP0 gene also contains a novel early promoter that is activated by DEX in mouse neuroblastoma cells (Wirth et al. 1992) (Fig. 2b, c). Expression of the cellular transcription factor, C/EBP-alpha, is stimulated by DEX, and C/EBP-alpha expression is necessary for DEX induction of bICP0 early promoter activity (Workman et al. 2009). C/EBP-alpha directly interacts with bICP0 early promoter sequences that are necessary for trans-activation by C/EBP-alpha. The bICP0 early promoter is also stimulated more than 100-fold by cellular transcription factors that regulate the cell cycle (E2F1 or E2F2) (Workman and Jones 2010). Two E2F

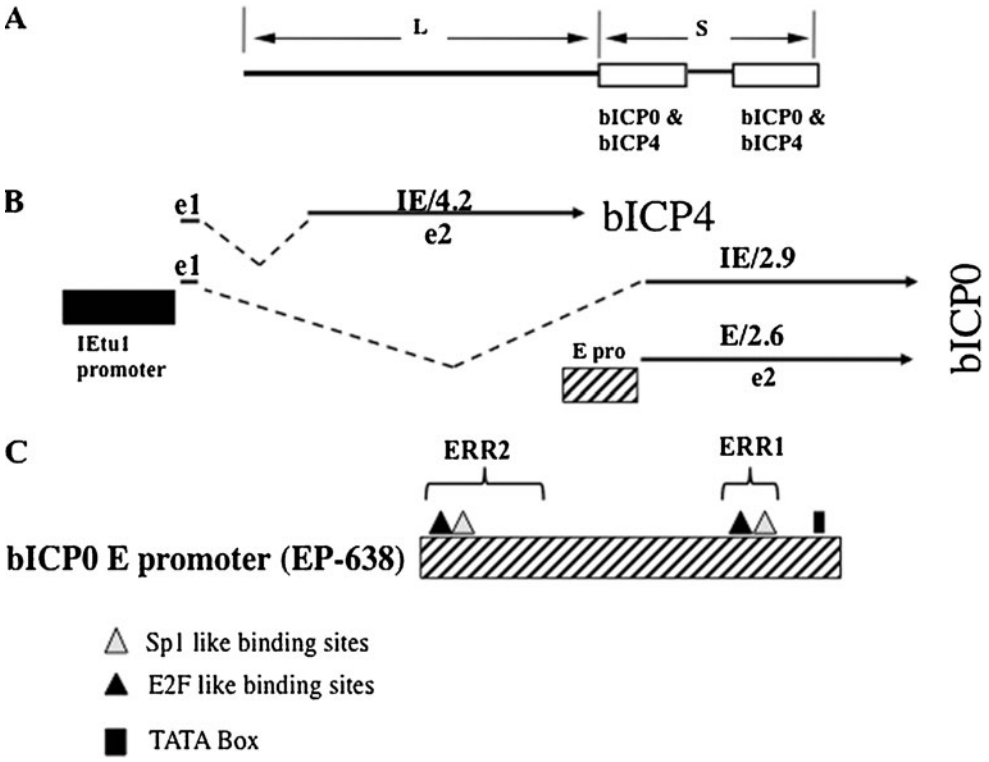

Fig. 2 Schematic of IEtu1 and bICP0 E promoter constructs used in this study. **a** Location of the unique long (*L*) and unique short (*S*) regions of the BHV-1 genome. The repeats are denoted by the *open rectangles*. Genes encoding bICP0 and bICP4 are present within the repeats. **b** Positions of bICP4 and bICP0 transcripts are shown. The immediate early transcription unit 1 (*IEtu1*) encodes bICP4 (*IE/4.2*) and bICP0 (*IE/2.9*) (Wirth et al. 1989, 1991). The IEtu1 promoter activates IE expression of IE/4.2 and IE/2.9 (denoted by the *black rectangle*). E/2.6 is the early transcript that encodes bICP0 and an early promoter activates expression of this transcript (Wirth et al. 1992). Exon 2 (*e2*) of bICP0 contains all of the protein coding sequences of bICP0. The *dashed lines* are intron sequences. **c** Schematic of the bICP0 early promoter regions (EP-638). EP-638 contains a 638-bp fragment that contains the E2F1 responsive region 1 (ERR1) and ERR2 (Workman and Jones 2010). ERR1 and ERR2 contain clusters of Sp1 and E2F like consensus binding sites. The location of the TATA box is also shown for reference to the location of ERR1 and ERR2

responsive regions (ERR) are located within the early promoter: one adjacent to the TATA box (ERR1) and one approximately 600 base pairs upstream from the TATA box (ERR2) (Fig. 2c). Mobility shift assays indicated that E2F transcription factors directly interact with ERR1 and ERR2. We suggest that activation of the bICP0 early promoter by cellular transcription factors induced by DEX is an early event during reactivation from latency. However, merely activating the bICP0 early promoter does not always lead to successful reactivation from latency. Finally, we predict that specific neuronal and/or viral factors are necessary to successfully complete reactivation from latency in a subset of latently infected neurons.

The LR gene encodes several factors that are expressed during latency

The LR gene encodes more than one product that may be important for the latency–reactivation cycle. For example, the LR gene contains two well-defined ORFs (ORF2 and ORF1; Fig. 1) and two reading frames that lack an initiating methionine (RF-B and RF-C). As a result of alternative splicing of polyA+ LR-RNA in TG of infected calves (Devireddy et al. 2003; Devireddy and Jones 1998), ORF2 can be fused with ORF1 protein coding sequences or RF-B. At 1 day after infection of calves and during latency, splicing occurs in TG such that ORF2 is intact. ORF2 protein expression, not merely LR-RNA expression, is required for inhibiting apoptosis in transiently transfected cells (Shen and Jones 2008) suggesting ORF2 plays an important role in the latency–reactivation cycle.

A yeast two-hybrid analysis revealed that ORF2 interacts with Notch1 and Notch3, components of the Notch signaling pathway (Workman et al. 2011). Notch receptor family members (Notch1–4) are membrane-tethered transcription factors that regulate numerous developmental and physiological processes (Bray 2006; Ehebauer et al. 2006). For example, Notch promotes

neuronal maintenance, development, and differentiation (Berezovska et al. 1999; Cornell and Eisen 2005; Justice and Jan 2002). Notch3 (Wang et al. 2007) and Notch1 (Naidr et al. 2003; Sade et al. 2004) promote cell survival by activating a protein kinase, AKT, that inhibits apoptosis. Conversely, other studies demonstrated that Notch family members induce apoptosis (Bray 2006; Ehebauer et al. 2006) suggesting Notch influences cell survival in a cell-type-dependent fashion. When the Notch receptor is engaged by one of its five transmembrane ligands (Jagged1, Jagged2, Delta-like1, Delta-like3, or Delta-like4), the Notch intracellular domain (ICD) is cleaved by specific proteases and subsequently translocates to the nucleus. In the nucleus, Notch ICD interacts with members of the CSL family of transcriptional factors, CBF1, Su(H), or Lag1 (also referred to as RBP*j*κ binding proteins), subsequently activating downstream genes.

The ability of ORF2 to interact with Notch1 and Notch3 may regulate cell survival and/or viral transcription as well as productive infection. In fact, Notch1, but not Notch3, enhances BHV-1 productive infection (Workman et al. 2011) and only Notch1 activates the BHV-1 immediate-early transcription unit 1 (IEtu1) and bICP0 early promoters: whereas Notch1 and Notch3 trans-activated the late glycoprotein C (gC) promoter. ORF2 interferes with the ability of Notch1 to trans-activate the bICP0 early promoter and Notch1 or Notch3 mediated activation of the gC promoter (Workman et al. 2011) suggesting this function is important for establishing and/or maintaining latency. Notch3 RNA levels are higher during DEX-induced reactivation from latency suggesting Notch family members stimulate productive infection during reactivation from latency. It remains to be seen whether ORF2 interferes with Notch signaling in general or selectively interferes with pathways that are important for viral replication.

An alternatively spliced LR transcript encodes a protein that contains most of ORF2 fused with ORF1 (Devireddy et al. 2003; Devireddy and Jones 1998). Using a bacterial two-hybrid assay, this fusion protein was shown to stably interact with the cellular transcription factor C/EBP-alpha (Meyer et al. 2007b). C/EBP-alpha RNA and protein levels increased in TG neurons during DEX-induced reactivation from latency. The finding that over-expression of C/EBP-alpha enhanced productive infection adds support for a role in C/EBP-alpha stimulating productive infection. Collectively, these studies suggest that ORF2 has two important functions: inhibiting apoptosis and interacting with specific cellular transcription factors that stimulate viral gene expression and productive infection.

Although the results from the LR mutant virus suggested that proteins encoded by the LR gene are necessary for the latency–reactivation cycle, non-protein coding functions within LR-RNA have also been identified. For example, the intact LR gene inhibits the ability of bICP0 to stimulate productive infection in a dose-dependent manner (Bratanich et al. 1992; Geiser et al. 2002). Insertion of three in-frame stop codons at the amino-terminus of the first ORF within the LR gene (ORF2) inhibited bICP0 repression with similar efficiency as the wt LR gene, suggesting expression of a LR protein is not required (Geiser et al. 2002). Since the LR gene is antisense to bICP0 coding sequences, we assumed LR-RNA hybridized to bICP0 RNA sequences and interfered with bICP0 expression. However, we were unable to obtain data indicating that antisense repression of bICP0 occurs. LR gene products also inhibit mammalian cell growth (Geiser and Jones 2005; Schang et al. 1996), and the cell growth inhibitory function of the LR gene maps to a 463-bp XbaI–PstI fragment (XP) (Geiser and Jones 2005). Sequences within the XP region have the potential to form stem-loop secondary structures suggesting this region encodes small non-coding RNAs (sncRNA). Two micro-RNAs located upstream of ORF2 are expressed during latency (Jaber et al. 2010b). These micro-RNAs or larger sncRNAs containing the micro-RNA sequences reduce bICP0 protein levels in transient transfection assays. It is not known whether the LR encoded micro-RNAs have other functions.

Identification of a novel transcript expressed in TG of latently infected neurons

A small ORF located within the LR promoter is designated ORF-E (Fig. 1b). ORF-E is antisense to the LR transcript, and downstream of bICP0 coding sequences, but does not overlap bICP0. The initiating methionine codon for ORF-E is located at nucleotide 697 and the terminating codon at nucleotide 297. The LR promoter contains multiple cis-acting motifs and has a 258-base-pair fragment (XbaI–SphI, Fig. 1a) that confers neuronal specific transcriptional activity to a heterologous promoter (Bratanich and Jones 1992; Delhon and Jones 1997; Jones et al. 1990). LR promoter sequences also contain a long AT-rich motif (40/53 nucleotides are A or T) that may promote ORF-E transcription. A transcript that encompasses ORF-E is expressed in productively infected bovine cells and in TG of latently infected calves (Inman et al. 2004). When ORF-E protein coding sequences are fused in frame with green fluorescent protein (GFP) sequences, GFP protein expression is detected in the nucleus of mouse or human neuroblastoma cells. In contrast, the ORF-E-GFP fusion protein is detected throughout rabbit skin cells. In transient trans-

fection assays, ORF-E promotes neurite formation in mouse neuroblastoma cells (Perez et al. 2007), which may promote the repair of damaged neurons following infection.

Comparison of the LR gene to HSV-1 latency-associated transcript (LAT)

The BHV-1 LR gene and HSV-1 LAT (latency-associated transcript) share certain functional properties even though they do not have strong sequence conservation. For example, LR-RNA and LAT are transcribed in an antisense direction of bICP0 and ICP0, respectively, and both are abundantly expressed during latency (Devireddy and Jones 1998; Hossain et al. 1995; Kutish et al. 1990; Rock et al. 1987; Schang and Jones 1997; Winkler et al. 2000a,b). Furthermore, the LR gene (Ciacci-Zanella et al. 1999; Shen and Jones 2008) and LAT (Ahmed et al. 2002; Inman et al. 2001a,b; Perng et al. 2000) inhibit apoptosis. Two sncRNAs encoded within LAT interfere with apoptosis (Shen et al. 2009), whereas ORF2 encoded by the LR gene inhibits apoptosis (Shen and Jones 2008). In addition, ORF-E (Inman et al. 2004), like AL1 (Perng et al. 2002a) and AL3 (Jaber et al. 2009), are transcribed in the opposite direction as the LR-RNA or LAT, respectively.

To test whether the LR gene restores spontaneous reactivation to a HSV-1 LAT deletion mutant (dLAT2903), a 2-kb fragment containing the LR promoter and LR coding sequences was inserted into the LAT locus of dLAT2903, and the recombinant virus designated CJLAT (Perng et al. 2002b). Insertion of the LR gene into the HSV-1 LAT locus restores high levels of spontaneous reactivation in the rabbit eye model and in explant-induced reactivation. Rabbits infected with CJLAT have higher levels of recurrent eye disease (stromal scarring and detached retinas). Further evidence for the expanded pathogenic properties of CJLAT came from the finding that CJLAT is more lethal in mice relative to LAT+ or LAT− strains of HSV-1. Insertion of the LR gene with stop codons into dLAT2903 generated a virus that behaved like the parental LAT null mutant adding further support that expression of LR proteins regulates the latency–reactivation cycle (Mott et al. 2003). Two additional anti-apoptosis genes (cpIAP and FLIP) restore wt levels of spontaneous reactivation from latency to dLAT2903 (Jin et al. 2005, 2008). Relative to wt HSV-1, these recombinants have reduced virulence. Although it appears that the anti-apoptosis functions of LAT and the LR gene are crucial for the latency–reactivation cycle, it seems clear that the LR gene contains virulence properties lacking in LAT.

Wild-type LR gene expression regulates the latency–reactivation cycle

In TG of latently infected calves, LR-RNA is abundantly expressed, and based on genetic evidence it seems clear that a protein or proteins encoded by the LR gene play an important role in the latency–reactivation cycle. It is not known whether ORF-E plays a role in latency–reactivation cycle. Based on what is currently known, we suggest the following model for how the LR gene regulates the latency–reactivation cycle in cattle (summarized in Fig. 3a). When the LR mutant virus infects a neuron that has the necessary cellular factors to support extensive viral gene expression and genome amplification ("permissive neuron"), this neuron frequently dies because of apoptosis, viral induced damage, and/or lymphocyte-mediated cytotoxicity. A permissive neuron can survive infection with wt BHV-1 because proteins, and perhaps regulatory RNAs, expressed by the LR gene inhibit productive infection and apoptosis. We predict that a permissive neuron has higher copies of viral genomes relative to a neuron that lacks cellular factors necessary for entry and/or viral genome amplification. Neurons that are refractile to viral entry, viral genome amplification, and/or viral gene expression are operationally defined as "non-permissive neurons". Permissive neurons would also appear to have a higher probability of reactivating from latency because they would likely contain fewer "repressive cellular factors" associated with each viral genome. Following a stressful stimulus, neurons that support reactivation from latency, but not those neurons

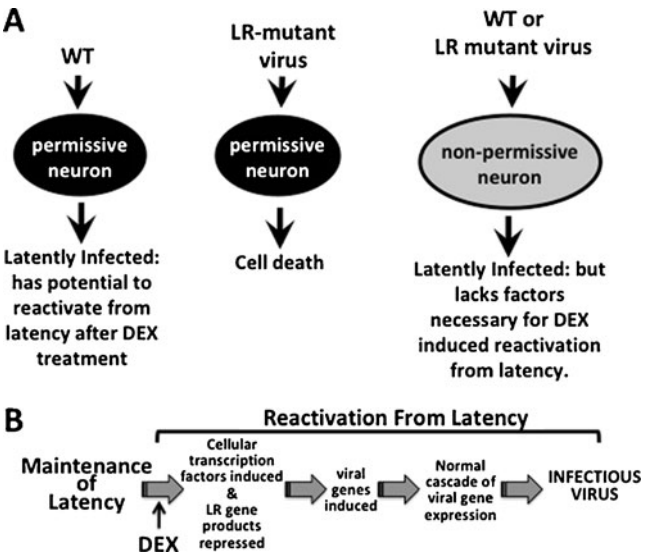

Fig. 3 Summary of events that occur in sensory neurons during the latency–reactivation cycle. **a** Schematic of proposed events that occur following infection of permissive versus non-permissive neurons. For details, see text. **b** Putative steps that occur during DEX-induced reactivation from latency. For details, see text

that do not support reactivation, would be predicted to express cellular factors that stimulate lytic cycle viral genes.

When wt BHV-1 or the LR mutant infects a non-permissive neuron, abundant viral gene expression and genome amplification does not occur. Consequently, latency is established regardless of LR gene expression. Latently infected non-permissive neurons do not reactivate from latency after DEX treatment because low levels of viral DNA are present and the neuronal environment does not support viral gene expression (reactivation from latency). In mice, distinct populations of neurons exist in TG and HSV-1 differentially infects these populations (Yang et al. 2000), suggesting that different neuronal populations exist in bovine TG. Calves infected with wt BHV-1, but not the LR mutant, contain neurons with high copies of viral DNA (Inman et al. 2002), adding support to the concept that permissive neurons exist in cattle.

Although expression of LR proteins regulates the latency–reactivation cycle, our studies suggest that non-protein coding RNAs encoded by the LR gene inhibit bICP0 expression (Geiser et al. 2002; Jaber et al. 2010b) and prevent cell growth (Geiser and Jones 2005). The ability of LR gene products to inhibit bICP0 expression promotes establishment and maintenance of latency. The significance of inhibiting cell growth is less clear-cut, but may be related to promoting neuronal survival because cell-cycle regulatory proteins are frequently expressed in neurons undergoing apoptosis (Freeman et al. 1994; Gill and Windebank 1998; Herrup and Busser 1995; Park et al. 1996, 1997a,b; Shirvan et al. 1997a,b, 1998). Furthermore, cell-cycle regulators can initiate apoptosis (Levkau et al. 1998; Meikrantz et al. 1994; Meikrantz and Schlegel 1996). Cyclin expression is induced in TG neurons during acute infection and reactivation from latency (Schang et al. 1996; Winkler et al. 2000a,b), indicating that there is a need for inhibiting the deleterious effects of cyclin expression in infected neurons. A cell-cycle-regulated transcription factor (E2F1) increases the efficiency of productive infection (Workman and Jones 2010) further suggests that inhibiting cell-cycle progression reduces the efficiency of productive infection. In summary, LR gene products are necessary for life-long latency in cattle.

Overview of putative events that occur during DEX-induced reactivation from latency

A working model for putative steps that lead to reactivation from latency is presented in Fig. 3b. During maintenance of latency, infectious virus is not detected, but LR gene products are abundantly expressed (reviewed in Jones 1998, 2003). This is important because ORF2 inhibits apoptosis and viral gene expression (Meyer et al. 2007b; Shen and Jones 2008; Workman et al. 2011). DEX initiates the exit from latency, in part, by inhibiting expression of all LR gene products (Jaber et al. 2010a; Jones 1998, 2003; Jones et al. 1990; Rock et al. 1992). Consequently, we predict that LR gene products promote the establishment and maintenance of latency, but have no direct role in reactivation from latency. Indirectly, LR gene products promote reactivation from latency by increasing the pool of neurons that survive acute infection.

Following DEX treatment, we predict that cellular transcription factors initiate lytic cycle viral gene expression (Fig. 3b). Recent unpublished studies have identified cellular transcription factors in TG that are stimulated by DEX, and these DEX-inducible transcription factors can activate certain viral promoters and productive infection. The normal cascade of BHV-1 gene expression may not occur during the escape from latency because (1) a late viral promoter (gC) is trans-activated by Notch1 or Notch3 (Workman et al. 2011) and (2) the bICP0 E promoter, but not its IE promoter (IEtu1), is consistently activated during reactivation from latency (Workman et al. 2009). With respect to HSV-1, several studies suggest that the normal cascade of viral gene expression may also be different during reactivation. For example, E gene expression and DNA replication is proposed to occur prior to IE gene expression (KosZ-Vnenchak et al. 1993; Nichol et al. 1996; Pesola et al. 2005; Tal-Singer et al. 1997). Another study concluded that expression of a late HSV-1 gene (VP16) promotes the early phases of reactivation of latency (Thompson et al. 2009). Activation of any viral gene during the early stages of reactivation from latency would appear to favor extensive viral transcription and perhaps the successful reactivation from latency.

Stimulation of BHV-1 regulatory proteins, for example bICP0 or bTIF, during reactivation from latency is predicted to restore the normal cascade of viral gene expression in a minor population of latently infection neurons. Under this scenario, all viral proteins would be expressed and infectious virus produced. It is difficult to imagine how successful reactivation from latency and spread to peripheral sites occur unless bICP0 protein expression occurs. Most neurons containing viral genomes do not appear to produce infectious virus (Rock et al. 1992), suggesting that certain neurons lack factors that are necessary for productive infection.

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References

Ahmed M, Lock M, Miller CG, Fraser NW (2002) Regions of the herpes simplex virus type 1 latency-associated transcript that protect cells from apoptosis in vitro and protect neuronal cells in vivo. J Virol 76:717–729

Berezovska O, McLean P, Knowles R, Lu FM, Lux SE, Hyman BT (1999) Notch1 inhibits neurite outgrowth in postmitotic primary neurons. Neuroscience 93:433–439

Bratanich AC, Jones CJ (1992) Localization of cis-acting sequences in the latency-related promoter of bovine herpesvirus 1 which are regulated by neuronal cell type factors and immediate-early genes. J Virol 66:6099–6106

Bratanich AC, Hanson ND, Jones C (1992) The latency-related gene of bovine herpesvirus 1 inhibits the activity of immediate-early transcription unit 1. Virology 191:988–991

Bray SJ (2006) Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol 7:678–689

Cantin EM, Hinton DR, Chen J, Openshaw H (1995) Gamma interferon expression during acute and latent nervous system infection by herpes simplex virus type 1. J Virol 69:4898–4905

Carter JJ, Weinberg AD, Pollard A, Reeves R, Magnuson JA, Magnuson NS (1989) Inhibition of T-lymphocyte mitogenic responses and effects on cell functions by bovine herpesvirus 1. J Virol 63:1525–1530

Ciacci-Zanella J, Stone M, Henderson G, Jones C (1999) The latency-related gene of bovine herpesvirus 1 inhibits programmed cell death. J Virol 73:9734–9740

Collins PL, McIntosh K, Chanock RM (2001) Respiratory syncytial virus. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin A, Roizman B, Straus SE (eds) Fields virology, 4th edn. Lippincott, Williams, and Wilkins, Philadelphia, pp 1443–1485

Cornell R, Eisen JS (2005) Notch in the pathway: the roles of Notch signalling in neural crest development. Semin Cell Dev Biol 16:663–672

Delhon G, Jones C (1997) Identification of DNA sequences in the latency related promoter of bovine herpes virus type 1 which are bound by neuronal specific factors. Virus Res 51:93–103

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

Devireddy LR, Jones C (1999) Activation of caspases and p53 by bovine herpesvirus 1 infection results in programmed cell death and efficient virus release. J Virol 73:3778–3788

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

Ehebauer M, Penelope P, Arias AM (2006) Notch, a universal arbiter of cell fate decisions. Science 314:1414–1415

Eskra L, Splitter GA (1997) Bovine herpesvirus-1 infects activated CD4+ lymphocytes. J Gen Virol 78:2159–2166

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

Frank GH (1984) Bacteria as etiologic agents in bovine respiratory disease. In: Loan RW (ed) Bovine respiratory disease. Texas A&M University Press, College Station, pp 384–392

Freeman RS, Estus S, Johnson EM Jr (1994) Analysis of cell cycle-related gene expression in postmitotic neurons: selective induction of Cyclin D1 during programmed cell death. Neuron 12:343–355

Fuchs M, Hubert P, Detterer J, Rziha HJ (1999) Detection of bovine herpesvirus type 1 in blood from naturally infected cattle by using a sensitive PCR that discriminates between wild-type virus and virus lacking glycoprotein E. J Clin Microbiol 37:2498–2507

Geiser V, Jones C (2005) The latency related gene encoded by bovine herpesvirus 1 encodes a small regulatory RNA that inhibits cell growth. J Neurovirol 11:563–570

Geiser V, Inman M, Zhang Y, Jones C (2002) The latency related (LR) gene of bovine herpes virus 1 (BHV-1) can inhibit the ability of bICP0 to activate productive infection. J Gen Virol 83:2965–2971

Gill JS, Windebank AJ (1998) Cisplatin-induced apoptosis in rat dorsal root ganglion neurons is associated with attempted entry into the cell cycle. J Clin Invest 101:2842–2850

Griebel P, Qualtiere L, Davis WC, Gee A, Bielefeldt Ohmann H, Lawman MJ, Babiuk LA (1987a) T lymphocyte population dynamics and function following a primary bovine herpesvirus type-1 infection. Viral Immunol 1:287–304

Griebel PJ, Qualtiere L, Davis WC, Lawman MJ, Babiuk LA (1987b) Bovine peripheral blood leukocyte subpopulation dynamics following a primary bovine herpesvirus-1 infection. Viral Immunol 1:267–286

Griebel P, Ohmann HB, Lawman MJ, Babiuk LA (1990) The interaction between bovine herpesvirus type 1 and activated bovine T lymphocytes. J Gen Virol 71:369–377

Halford WP, Gebhardt BM, Carr DJ (1996) Persistent cytokine expression in trigeminal ganglion latently infected with herpes simplex virus type 1. J Immunol 157:3542–3549

Hariharan MJ, Nataraj C, Srikumaran S (1993) Down regulation of murine MHC class I expression by bovine herpesvirus 1. Viral Immunol 6:273–284

Henderson G, Zhang Y, Jones C (2005) 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 86:2697–2702

Herrup K, Busser JC (1995) The induction of multiple cell cycle events precedes target-related neuronal death. Development 121:2385–2395

Highlander SK (2001) Molecular genetic analysis of virulence in *Mannheimia (Pasteurella) haemolytica*. Front Biosci :D1128–1150

Highlander SK, Fedorova MD, Dusek DM, Panciera R, Alvarez LE, Renehart C (2000) Inactivation of *Pasteurella (Mannheimia) haemolytica* leukotoxin causes partial attenuation of virulence in a calf challenge model. Infect Immun 68:3916–3922

Hinkley S, Hill AB, Srikumaran S (1998) Bovine herpesvirus-1 infection affects the peptide transport activity in bovine cells. Virus Res 53:91–96

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

Inman M, Perng G-C, Henderson G, Ghiasi H, Nesburn AB, Wechsler SL, Jones C (2001a) Region of herpes simplex virus type 1 latency-associated transcript sufficient for wild-type spontaneous reactivation promotes cell survival in tissue culture. J Virol 75:3636–3646

Inman M, Lovato L, Doster A, Jones C (2001b) 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, Lovato L, Doster A, Jones C (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, Zhou J, Webb H, Jones C (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

Jaber T, Henderson G, Li S, Perng G-C, Carpenter D, Wechsler S, Jones C (2009) Identification of a novel herpes simplex virus

type 1 (HSV-1) transcript and protein (AL3) expressed during latency. J Gen Virol 90:2342–2352

Jaber T, Workman A, Jones C (2010a) Small non-coding RNAs encoded within the bovine herpesvirus 1 latency related gene can reduce steady state levels of infected cell protein 0 (bICP0). J Virol 84:6297–6307

Jaber T, Workman A, Jones C (2010b) Small non-coding RNAs encoded within the bovine herpesvirus 1 latency related gene can reduce steady state levels of infected cell protein 0 (bICP0). J Virol 84

Jiang Y, Hossain A, Winkler MT, Holt T, Doster A, Jones C (1998) A protein encoded by the latency-related gene of bovine herpesvirus 1 is expressed in trigeminal ganglionic neurons of latently infected cattle and interacts with cyclin-dependent kinase 2 during productive infection. J Virol 72:8133–8142

Jiang Y, Inman M, Zhang Y, Posadas NA, Jones C (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

Jin L, Perng G-C, Nesburn AB, Jones C, Wechsler SL (2005) The baculovirus inhibitor of apoptosis gene (cpIAP) can restore reactivation of latency to a herpes simplex virus type 1 that does not express the latency associated transcript (LAT). J Virol 79:12286–12295

Jin L, Carpenter D, Moerdyk-Schauwecker M, Vanarsdall AL, Osorio N, Hsiang C, Jones C, Wechsler SL (2008) Cellular FLIP can substitute for the herpes simplex virus type 1 LAT gene to support a wild type virus reactivation phenotype in mice. J Neurovirol 14:389–400

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 Microbiol Rev 16:79–95

Jones C (2009) Regulation of innate immune responses by bovine herpesvirus 1 and infected cell protein 0. Viruses 1:255–275

Jones C, Delhon G, Bratanich A, Kutish G, Rock D (1990) Analysis of the transcriptional promoter which regulates the latency-related transcript of bovine herpesvirus 1. J Virol 64:1164–1170

Jones C, Newby TJ, Holt T, Doster A, Stone M, Ciacci-Zanella J, Webster CJ, Jackwood MW (2000) Analysis of latency in cattle after inoculation with a temperature sensitive mutant of bovine herpesvirus 1 (RLB106). Vaccine 18:3185–3195

Justice NJ, Jan YN (2002) Variations on the Notch pathway in neural development. Curr Opin Neurobiol 12:64–70

Khanna KM, Bonneau RH, Kinchington PR, Hendricks RL (2003) Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia. Immunity 18:593–603

Knickelbein JE, Khanna KM, Yee MB, Baty CJ, Kinchington PR, Hendricks RL (2008) Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency. Science 322:268–272

Koppers-Lalic EA, Reits EAJ, Ressing ME, Lipinska AD, Abele R, Koch J, Rezende MM, Admiraal P, van Leeuwen D, Bienkowsaka-Szewczyc K, Mettenleiter TC, Rijsewijk FAM, Tampe R, Neefjes J, Wiertz EJHJ (2005) Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing. Proc Natl Acad Sci USA 102:5144–5149

KosZ-Vnenchak JJ, Coen DM, Knipe DM (1993) Evidence for a novel regulatory pathway for herpes simplex virus gene expression in trigeminal ganglion neurons. J Virol 67:5383–5393

Kutish G, Mainprize T, Rock D (1990) Characterization of the latency-related transcriptionally active region of the bovine herpesvirus 1 genome. J Virol 64:5730–5737

Levkau B, Koyama H, Raines EW, Clurman BE, Herren B, Orth K, Roberts JM, Ross R (1998) Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Mol Cell 1:553–563

Liu T, Tang Q, Hendricks RL (1996) Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. J Virol 70:264–271

Liu T, Khanna KM, Chen X, Fink DJ, Hendricks RL (2000) CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons. J Exp Med 191:1459–1466

Liu T, Khanna KM, Carriere BN, Hendricks RL (2001) Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons. J Virol 75:11178–11184

Lovato L, Inman M, Henderson G, Doster A, Jones C (2003) Infection of cattle with a bovine herpesvirus 1 (BHV-1) strain that contains a mutation in the latency related gene leads to increased apoptosis in trigeminal ganglia during the transition from acute infection to latency. J Virol 77:4848–4857

Meikrantz W, Schlegel R (1996) Suppression of apoptosis by dominant negative mutants of cyclin-dependent protein kinases. J Biol Chem 271:10205–10209

Meikrantz W, Geisselbrecht S, Tam SW, Schlegel R (1994) Activation of cyclin A-dependent protein kinases during apoptosis. Proc Natl Acad Sci USA 91:3754–3758

Meyer F, Perez S, Jiang Y, Zhou Y, Henderson G, Jones C (2007a) Identification of a novel protein encoded by the latency-related gene of bovine herpesvirus 1. J Neurovirol 13:569–578

Meyer F, Perez S, Geiser V, Sintek M, Inman M, Jones C (2007b) 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, Bratanich AC, Carpenter D, O'Hare P (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, Walker S, Hayes S, O'Hare P (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

Mott K, Osorio N, Jin L, Brick D, Naito J, Cooper J, Henderson G, Inman M, Jones C, Wechsler SL, Perng G-C (2003) The bovine herpesvirus 1 LR ORF2 is crucial for this gene's ability to restore the high reactivation phenotype to a Herpes simplex virus-1 LAT null mutant. J Gen Virol 84:2975–2985

Mweene AS, Okazaki K, Kida H (1996) Detection of viral genome in non-neural tissues of cattle experimentally infected with bovine herpesvirus 1. Jpn J Vet Res 44:165–174

Naidr P, Somasundaram K, Krishna S (2003) Activated Notch1 inhibits p53-induced apoptosis and sustains transformation by human papilloma virus type 16 E6 and E7 oncogenes through a PI3K-PKB/Akt-dependent pathway. J Virol 77:7106–7112

Nataraj C, Eidmann S, Hariharan MJ, Sur JH, Perry GA, Srikumaran S (1997) Bovine herpesvirus 1 downregulates the expression of bovine MHC class I molecules. Viral Immunol 10:21–34

Nichol PF, Chang JY, Johnson EM Jr, Olivo PD (1996) Herpes simplex virus gene expression in neurons: viral DNA synthesis is a critical regulatory event in the branch point between lytic and latent pathways. J Virol 70:5476–5486

Park DS, Farinelli SE, Greene LA (1996) Inhibitors of cyclin-dependent kinases promote survival of post-mitotic neuronally differentiated PC12 cells and sympathetic neurons. J Biol Chem 271:8161–8169

Park DS, Levine B, Ferrari G, Greene LA (1997a) Cyclin dependent kinase inhibitors and dominant negative cyclin dependent kinase

4 and 6 promote survival of NGF-deprived sympathetic neurons. J Neurosci 17:8975–8983

Park DS, Morris EJ, Greene LA, Geller HM (1997b) G1/S cell cycle blockers and inhibitors of cyclin-dependent kinases suppress camptothecin-induced neuronal apoptosis. J Neurosci 17:1256–1270

Perez S, Inman M, Doster A, Jones C (2005) Latency-related gene encoded by bovine herpesvirus 1 promotes virus growth and reactivation from latency in tonsils of infected calves. J Clin Microbiol 43:393–401

Perez S, Meyer F, Henderson G, Jiang Y, Sherman S, Doster A, Inman M, Jones C (2007) A protein encoded by the bovine herpesvirus 1 ORF E gene induces neurite-like morphological changes in mouse neuroblastoma cells and is expressed in trigeminal ganglionic neurons. J Neurovirol 13:139–149

Perng G-C, Jones C, Ciacci-Zanella J, Stone M, Henderson G, Yukht A, Slanina SM, Hoffman FM, Ghiasi H, Nesburn AB, Wechsler SL (2000) Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript (LAT). Science 287:1500–1503

Perng G-C, Maguen B, Jing L, Mott KR, Osorio N, Slanina SM, Yukht A, Ghiasi H, Nesburn AB, Inman M, Henderson G, Jones C, Wechsler SL (2002a) A novel herpes simplex virus type 1 (HSV-1) transcript (AL-RNA) antisense to the 5' end of LAT (latency associated transcript) produces a protein in infected rabbits. J Virol 76:8003–8010

Perng G-C, Maguen B, Jin L, Mott KR, Osorio N, Slanina SM, Yukht A, Ghiasi H, Nesburn AB, Inman M, Henderson G, Jones C, Wechsler SL (2002b) A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. J Virol 76:1224–1235

Pesola JM, Zhu J, Knipe DM, Coen DM (2005) Herpes simplex virus 1 immediate-early and early gene expression during reactivation from latency under conditions that prevent infectious virus production. J Virol 79:14516–14525

Prbhakaran K, Sheridan BS, Kinchington PR, Khanna KM, Decman V, Lathrop K, Hendricks RL (2005) Sensory neurons regulate the effector functions of CD8+ T cells in controlling HSV-1 latency ex vivo. Immunity 23:515–523

Rice SA, Long MC, Lam V, Schaffer PA, Spencer CA (1995) Herpes simplex virus immediate-early protein ICP22 is required for viral modification of host RNA polymerase II and establishment of the normal viral transcription program. J Virol 69:5550–5559

Rice JA, LC-M, Hodgins DC, Shewen PE (2008) *Mannheimia haemolytica* and bovine respiratory disease. Anim Health Res Rev 8:117–128

Rivera-Rivas JJ, Kisiela D, Czuprynski CJ (2009) Bovine herpesvirus type 1 infection of bovine bronchial epithelial cells increases neutrophil adhesion and activation. Vet Immunol Immunopathol 131:167–176

Rock DL, Beam SL, Mayfield JE (1987) Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. J Virol 61:3827–3831

Rock D, Lokensgard J, Lewis T, Kutish G (1992) Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. J Virol 66:2484–2490

Sade H, Krishna S, Sarin A (2004) The anti-apoptotic effect of Notch-1 requires p56lck-dependent, AKT/PKB-mediated signaling in T cells. J Biol Chem 279:2937–2944

Saira K, Jones C (2009) 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 83:3977–3981

Saira K, Zhou Y, Jones C (2007) 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 81:3077–3086

Schang L, Jones C (1997) Analysis of bovine herpesvirus 1 transcripts during a primary infection of trigeminal ganglia of cattle. J Virol 71:6786–6795

Schang LM, Hossain A, Jones C (1996) The latency-related gene of bovine herpesvirus 1 encodes a product which inhibits cell cycle progression. J Virol 70:3807–3814

Shen W, Jones C (2008) Open reading frame 2, encoded by the latency-related gene of bovine herpesvirus 1, has antiapoptotic activity in transiently transfected neuroblastoma cells. J Virol 82:10940–10945

Shen W, Silva MS, Jaber T, Vitvitskaia O, Li S, Henderson G, Jones C (2009) Two small RNAs encoded within the first 1.5 kb of the herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) can inhibit productive infection, and cooperate to inhibit apoptosis. J Virol 90:9131–9139

Shimeld C, Whiteland JL, Nicholls SM, Grinfeld E, Easty DL, Gao H, Hill TJ (1995) Immune cell infiltration and persistence in the mouse trigeminal ganglion after infection of the cornea with herpes simplex virus type 1. J Neuroimmunol 61:7–16

Shimeld C, Whiteland JL, Williams NA, Easty DL, Hill TJ (1996) Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: an in vivo study of virus antigen and immune cell infiltration. J Gen Virol 77:2583–2590

Shimeld C, Whiteland JL, Williams NA, Easty DL, Hill TJ (1997) Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1. J Gen Virol 78:3317–3325

Shirvan A, Ziv I, Barzilai A, Djaldeti R, Zilkh-Falb R, Michlin T, Melamed E (1997a) Induction of mitosis-related genes during dopamine-triggered apoptosis in sympathetic neurons. J Neural Transm (Suppl 50):67–78

Shirvan A, Ziv I, Machlin T, Zilkha-Falb R, Melamed E, Barzilai A (1997b) Two waves of cyclin B and proliferating cell nuclear antigen expression during dopamine-triggered neuronal apoptosis. J Neurochem 69:539–549

Shirvan A, Ziv I, Zilkha-Falb R, Machlyn T, Barzilai A, Melamed E (1998) Expression of cell cycle-related genes during neuronal apoptosis: is there a distinct pattern? Neurochem Res 23:767–777

Smith CA, Bates P, Rivera-Gonzalez R, Gu B, DeLuca NA (1993) ICP4, the major transcriptional regulatory protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. J Virol 67:4676–4687

Songer JG, Post KW (2005) The Genera *Mannheimia* and *Pasteurella*. In: Duncan L (ed) Veterinary microbiology: bacterial and fungal agents of animal disease. Elsevier Saunders, St Louis

Srikumaran S, Ambagela A, Kelling CL (2007) Immune evasion by pathogens of bovine respiratory disease complex. Anim Health Res Rev 8:215–229

Tal-Singer R, Lasner TM, Podrzucki W, Skokotas A, Leary JJ, Berger SL, Frazer NW (1997) Gene expression during reactivation of herpes simplex virus type 1 from latency in the peripheral nervous system is different from that during lytic infection of tissue cultures. J Virol 71:5268–5276

Theil D, Derfuss T, Paripovic I, Herberger S, Meinl E, Schueler O, Strupp M, Arbusow V, Brandt T (2003) Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response. Am J Pathol 163:2179–2184

Thompson RL, Preston CM, Sawtell NM (2009) De novo synthesis of VP16 coordinates the exit form HSV latency in vivo. Plos Pathog 5:1–12

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

Wang T, Holt CM, Xu C, Ridley C, Jones R, Baron M, Trump D (2007) Notch 3 activation modulates growth behavior and cross talks to Wnt/TCF signalling pathway. Cell Signal 19:2458–2467

Winkler MT, Doster A, Jones C (1999) Bovine herpesvirus 1 can infect CD4(+) T lymphocytes and induce programmed cell death during acute infection of cattle. J Virol 73:8657–8668

Winkler MT, Schang LS, Doster A, Holt T, Jones C (2000a) Analysis of cyclins in trigeminal ganglia of calves infected with bovine herpesvirus-1. J Gen Virol 81:2993–2998

Winkler MTC, Doster A, Jones C (2000b) Persistence and reactivation of bovine herpesvirus 1 in the tonsil of latently infected calves. J Virol 74:5337–5346

Winkler MT, Doster A, Sur JH, Jones C (2002) Analysis of bovine trigeminal ganglia following infection with bovine herpesvirus 1. Vet Microbiol 86:139–155

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

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

Wirth UV, Fraefel C, Vogt B, Vlcek C, Paces V, Schwyzer M (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

Workman A, Jones C (2010) Bovine herpesvirus 1 productive infection and bICP0 early promoter activity are stimulated by E2F1. J Virol 84:6308–6317

Workman A, Perez S, Doster A, Jones C (2009) Dexamethasone treatment of calves latently infected with bovine herpesvirus 1 (BHV-1) leads to activation of the bICP0 early promoter, in part by the cellular transcription factor C/EBP-alpha. J Virol 83:8800–8809

Workman A, Sinani D, Pittayakhajonwut D, Jones C (2011) A protein (ORF2) encoded by the latency related gene of bovine herpesvirus 1 interacts with Notch1 and Notch3. J Virol 85:2536–2546

Yang L, Voytek CC, Margolis TP (2000) Immunohistochemical analysis of primary sensory neurons latently infected with herpes simplex virus type 1. J Virol 74:209–217

Zecchinon L, Fett T, Desmecht D (2005) How *Mannheimia haemolytica* defeats host defense through a kiss of death mechanism. Vet Res 36:133–156