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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- α , 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

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) (Figure 1a). LR-RNA is transcribed antisense with respect to the IE and E gene transcript (IE2.9/E2.6) that encodes bICP0 (Figure 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) (Figure 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.

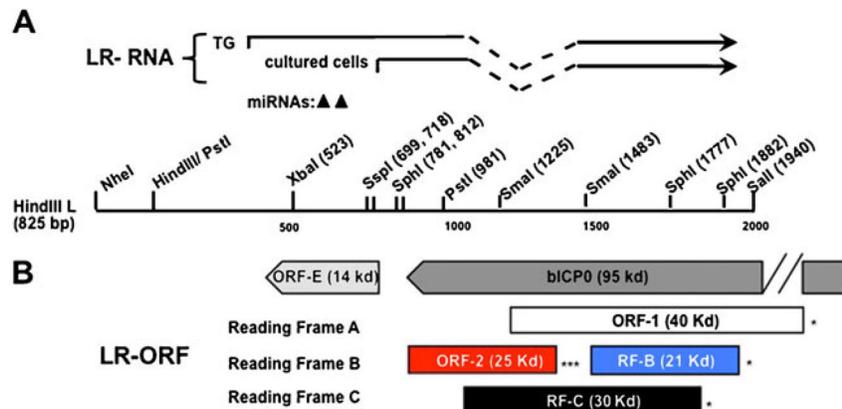


Figure 1. Schematic of the LR gene and surrounding genes. **a.)** Partial restriction map of LR gene, location of LR-RNA, and position of two miRNAs located within the LR gene. The start sites for LR transcription during latency and productive infection were previously described (Devireddy and Jones 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 (Figure 2b), this suggests the I_{ETu1} 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) (Figure 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 responsive regions (ERR) are located

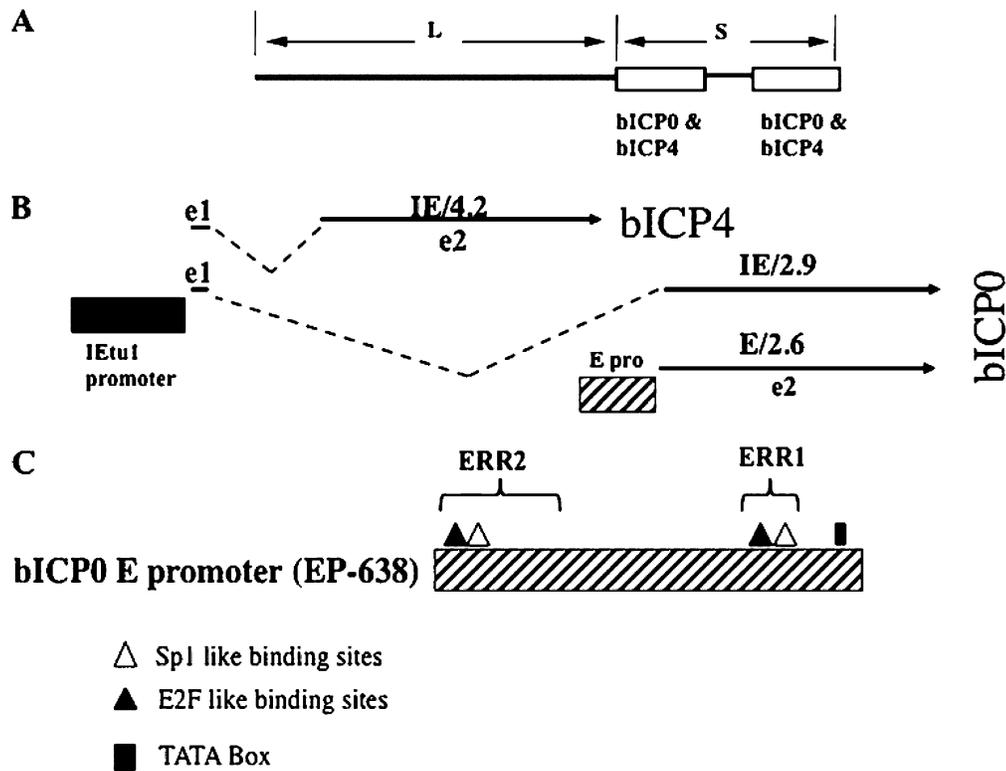


Figure 2. Schematic of IETu1 and bICP0 E promoter constructs used in this study. a Location of the unique long (L) and unique short (S) regions of the BHV-1 genome. The repeats are denoted by the *open rectangles*. Genes encoding bICP0 and bICP4 are present within the repeats. b Positions of bICP4 and 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.

within the early promoter: one adjacent to the TATA box (ERR1) and one approximately 600 base pairs upstream from the TATA box (ERR2) (Figure 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; Figure 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 RBPjk 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 (IEt1) 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 overexpression 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 microRNAs 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 (Figure 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, Figure 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 transfection 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. 2000*a,b*). Furthermore, the LR gene (Ciacci-Zanella et al. 1999; Shen and Jones 2008) and LAT (Ahmed et al. 2002; Inman et al. 2001*a,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. 2002*a*) 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. 2002*b*). 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 Figure 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 that do not support reactivation, would be predicted to express cellular factors that stimulate lytic cycle viral genes.

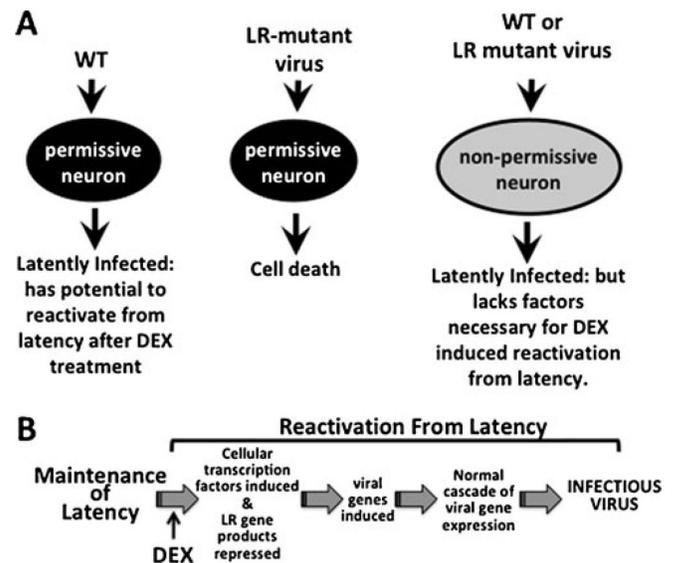


Figure 3. Summary of events that occur in sensory neurons during the latency-reactivation cycle. **a.)** Schematic of proposed events that occur following infection of permissive versus non-permissive neurons. For details, see text. **b.)** Putative steps that occur during DEX-induced reactivation from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

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 (re-activation 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 Figure 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 (Figure 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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