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REVIEW

Bovine Herpes Virus 1 (BHV-1) and Herpes Simplex Virus Type 1 (HSV-1) Promote Survival of Latently Infected Sensory Neurons, in Part by Inhibiting Apoptosis

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Abstract: α-Herpesvirinae subfamily members, including herpes simplex virus type 1 (HSV-1) and bovine herpes virus 1 (BHV-1), initiate infection in mucosal surfaces. BHV-1 and HSV-1 enter sensory neurons by cell-cell spread where a burst of viral gene expression occurs. When compared to non-neuronal cells, viral gene expression is quickly extinguished in sensory neurons resulting in neuronal survival and latency. The HSV-1 latency associated transcript (LAT), which is abundantly expressed in latently infected neurons, inhibits apoptosis, viral transcription, and productive infection, and directly or indirectly enhances reactivation from latency in small animal models. Three anti-apoptosis genes can be substituted for LAT, which will restore wild type levels of reactivation from latency to a LAT null mutant virus. Two small non-coding RNAs encoded by LAT possess anti-apoptosis functions in transfected cells. The BHV-1 latency related RNA (LR-RNA), like LAT, is abundantly expressed during latency. The LR-RNA encodes a protein (ORF2) and two microRNAs that are expressed in certain latently infected neurons. Wild-type expression of LR gene products is required for stress-induced reactivation from latency in cattle. ORF2 has anti-apoptosis functions and interacts with certain cellular transcription factors that stimulate viral transcription and productive infection. ORF2 is predicted to promote survival of infected neurons by inhibiting apoptosis and sequestering cellular transcription factors which stimulate productive infection. In addition, the LR encoded microRNAs inhibit viral transcription and apoptosis. In summary, the ability of BHV-1 and HSV-1 to interfere with apoptosis and productive infection in sensory neurons is crucial for the life-long latency-reactivation cycle in their respective hosts.

Keywords: alpha-herpesviruses, latency in sensory neurons, apoptosis, non-coding RNAs

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α-Herpesvirinae Subfamily Members are Important Pathogens in their Respective Hosts

Herpes simplex virus type 1 (HSV-1) and bovine herpes virus 1 (BHV-1) are both important pathogens in their respective natural hosts and both are α-herpesvirinae subfamily members. For example, HSV-1 is the cause of one of the most frequent and serious viral eye infections in the United States, with over 400,000 affected individuals.[1] Following primary infection of the eye, latency is established in sensory neurons within trigeminal ganglia (TG).[2,3] HSV-1 reactivates sporadically from TG and the infectious virus can be detected on surfaces of the eye, where it can cause recurrent ocular disease. Reactivation from latency is necessary for recurrent ocular HSV-1 infections.[4,5] Long-term oral acyclovir treatment only reduces ocular HSV-1 recurrences by 41%.[6] Herpes simplex virus type 2 (HSV-2) is the cause of the most common sexually transmitted disease, and sporadic recurrent genital lesions occur periodically. Two genital herpes vaccine trials failed[7,8] indicating there is a need for new and effective therapies that will reduce the incidence of recurrent HSV-1 and HSV-2 disease.

Bovine herpes virus 1 (BHV-1) is an important pathogen of cattle as it induces clinical signs in the upper respiratory tract of cattle and is immunesuppressive. BHV-1 establishes latency in sensory neurons, but periodically reactivates from latency, and thus is widespread in cattle.[2,9–11] BHV-1 infection inhibits cell-mediated immunity,[12–15] $CD8^+$ T cell recognition of infected cells,[16–19] and induces apoptosis in $CD4^+$ T cells.[20,21] Two viral regulatory proteins, bICP0 and bICP27, inhibit interferon dependent transcription.[10,22–25] Infection also erodes mucosal surfaces of the upper respiratory tract, which can allow bacterial pathogens to colonize the lower respiratory tract.[26–28]

Acute Infection Results in High Levels of Infectious Virus and Apoptosis

Binding and entry of HSV-1 and BHV-1 to mammalian cells are mediated by viral glycoproteins and cellular factors.[29–31] A cellular receptor (HveA or HVEM) is primarily expressed in activated T cells and belongs to the tumor necrosis factor receptor family.[32] Entry of HSV-1 into epithelial and fibroblasts is mediated by another membrane glycoprotein, HveB or HveC.[33] HveC is an entry mediator for HSV-1 and BHV-1 and is abundantly expressed in neurons. Additionally, soluble HveC blocks viral entry in neuronal-like cell lines.[33] After uncoating, the viral genome enters the nucleus and productive infection is initiated.

HSV-1 and BHV-1 gene expression is tightly regulated in three distinct phases during productive infection of cultured cells: immediate early (IE), early (E), or late (L).[34] IE RNA expression does not require protein synthesis and is stimulated by VP16, a viral structural protein.[35] E gene expression requires at least one IE protein, and E genes encode nonstructural proteins that stimulate viral DNA replication. L gene expression is maximal after viral DNA replication, requires IE protein production, and L proteins comprise the virion particle. Although a vigorous immune response leads to viral clearance following primary infection, BHV-1 and HSV-1 establish a life-long latent infection in ganglionic sensory neurons, primarily TG, or sacral dorsal root ganglia.[2,3,9,36] Approximately 40% of sensory neurons appear to harbor viral genomes during latency.[37–41]

Five HSV-1 IE genes encode ICP0, ICP4, ICP22, ICP27, or ICP47. ICP4[42–45] and ICP27[46–48] are required for virus growth in tissue culture. ICP4 represses IE gene expression[44,49–53] but activates E or L gene expression by interacting with RNA polymerase II transcription factors and specifically binding viral DNA.[49,54] ICP27 redistributes small nuclear ribonucleoprotein complexes, interferes with splicing of IE transcripts, and promotes E and L poly A site selection.[55–58] ICP47 prevents transport of antigenic peptides into the endoplasmic reticulum[59] and inhibits $CD8^+$ T cell responses.[60] ICP22 enhances viral gene expression, in part by modifying RNA polymerase II.[61] ICP0 increases steady-state levels of viral mRNA and stimulates all viral promoters.[62] ICP0 also binds several cellular proteins: (1) elongation factor 1-α[63]; (2) cyclin D3[64]; (3) an ubiquitin-specific protease[65,66]; and (4) promyelocytic leukemia (PML) protein.[67–69] Interactions between ICP0 and chromatin-remodeling enzymes activate viral transcription by multiple mechanisms, including sequestering histone deacetylase (HDAC) inhibitors.[70,71] Secondly, HSV-1 ICP0 interacts with HDAC2[72] and blocks histone deacetylation to stimulate viral gene expression.[73,74] ICP0 also alters a complex that inhibits

gene expression (REST/CoREST/HDAC repressor complex).[73] Since ICP0 can remove histones from viral chromatin during productive infection,[75] ICP0 may have similar functions during reactivation from latency. These activities of ICP0 promote virus replication in differentiated cells.[76] BHV-1 encoded ICP0 (bICP0) has similar functions as ICP0.[10]

Viral infection routinely leads to apoptosis in cultured cells.[77–80] Killing of infected cells by apoptosis in vivo can reduce inflammation, alter immune recognition, reduce burst size, and thus prevent virus spread. Premature apoptosis of infected cells limits production of infectious virus and limits viral spread. Members of the α-herpesvirinae subfamily induce apoptosis after infection of cultured cells.[81–86] HSV-1[83,84,87–89] and BHV-1[90] can also inhibit apoptosis in a cell type dependent manner after infection of cultured cells. HSV can induce DNA damage, and consequently apoptosis, even in the absence of productive infection.[91–95] Two viral proteins, $U_S1.5$ and U_L13, activate caspase 3 in the absence of other viral proteins, indicating these viral proteins play an important role during virus mediated apoptosis.[96] Finally, ICP0 is also a trigger for apoptosis in the context of productive infection, in part because it activates viral gene expression.

HSV-1 encodes several proteins (ICP27, U_s3, U_s5, gJ, gD, and LAT) that have anti-apoptosis activity.[83–85,87,88,97–105] U_s3 is a serine/threonine protein kinase that inhibits cleavage and activation of the pro-apoptotic Bcl-2 family member, Bad. U_s3 protein expression in cultured cells, in the absence of other viral proteins, inhibits caspase 3 activation, a crucial executioner caspase that commits cells to apoptosis. As expected, U_S3 inhibits the pro-apoptotic activity of $U_S1.5$ and U_L13 by blocking caspase 3 activation.[96] These anti-apoptotic genes play an important role in the pathogenic properties of HSV-1.

Viral Genes Expressed During Latency Regulate the Latency-Reactivation Cycle

The HSV-1 latency associated transcript is abundantly expressed during latency and regulates the latency-reactivation cycle

The HSV-1 latency associated transcript (LAT) is abundantly expressed in sensory gangionic neurons of mice, rabbits, or humans that are latently infected.[106–114] LAT is predominantly expressed in the nucleus of latently infected neurons suggesting it is a non-protein coding regulatory RNA. LAT is antisense to ICP0 and overlaps ICP0 mRNA sequences (Fig. 1B), suggesting LAT inhibits ICP0 expression by an antisense mechanism. Although the ability of LAT to repress ICP0 expression may be important, LAT sequences that promote spontaneous reactivation in a rabbit ocular model of infection do not overlap ICP0 mRNA sequences.[115]

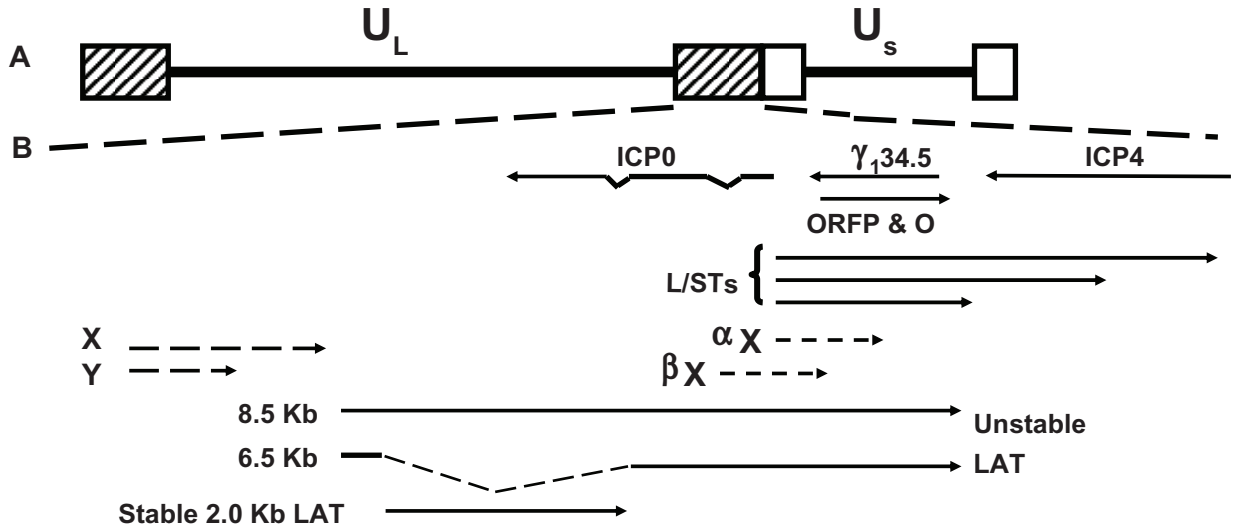

Figure 1. Location of genes within the HSV-1 repeats. (Panel **A**) U_L and U_S denote the unique sequences of the long (L) and short (S) components of the genome. The boxes depict repeat sequences. (Panel **B**) Transcription map of the repeat region. Location and orientation of LAT,[111,112] ICP0, $\alpha_1$34.5,[253,254] ORFP,[150] L/STs[255] are indicated by solid lines.
Note: Partially mapped transcripts (αX and αX) are denoted by dashed arrows.[256,257]

Splicing of the 8.5 kb LAT transcript yields a stable 2 kb LAT and an unstable 6.5 kb LAT (Fig. 1B).[107,111,116] Correct splicing of the 2 kb LAT is necessary for establishment and maintenance of latency.[117,118] In general, the 2 kb LAT is not capped, is poly A-, appears to be circular, and is a stable intron.[119,120] A subset of LAT is detected in the cytoplasm[97,121,122] and is associated with polyribosomes or splicing factors.[97,123] Small non-coding RNAs can regulate gene expression,[124,125] promote neuronal differentiation,[126] or inhibit apoptosis[127] suggesting LAT is a non-coding regulatory RNA.

A study by Umbach et al[128] concluded that LAT is a microRNA (miRNA) precursor which encodes four miRNAs, two within LAT promoter sequences (Fig. 2A and B). LAT miR-H6, reduces ICP4 protein steady state levels but not ICP4 RNA levels. Protein levels, not RNA levels, of ICP0 are inhibited by the LAT miRNA, miR-H2-3p. Within the first 1.5 kb of LAT coding sequences, two small RNAs (sRNAs), LAT sRNA1 and sRNA2, were identified (Fig. 2B). The sRNAs are larger than mature miRNAs (typically 23 nucleotides long) and the sequence of both possess extensive secondary structure. The sRNAs can be detected in TG of mice latently infected with wild-type HSV-1, but not in TG of mice latently infected with a LAT null mutant.[79,129] LAT sRNA2, but not LAT sRNA1, reduced ICP4 protein levels in transient transfection assays. Both LAT sRNAs inhibit productive infection in mouse neuroblastoma cells, however LAT sRNA1 inhibited productive infection more efficiently than LAT sRNA2.[130] Collectively, these

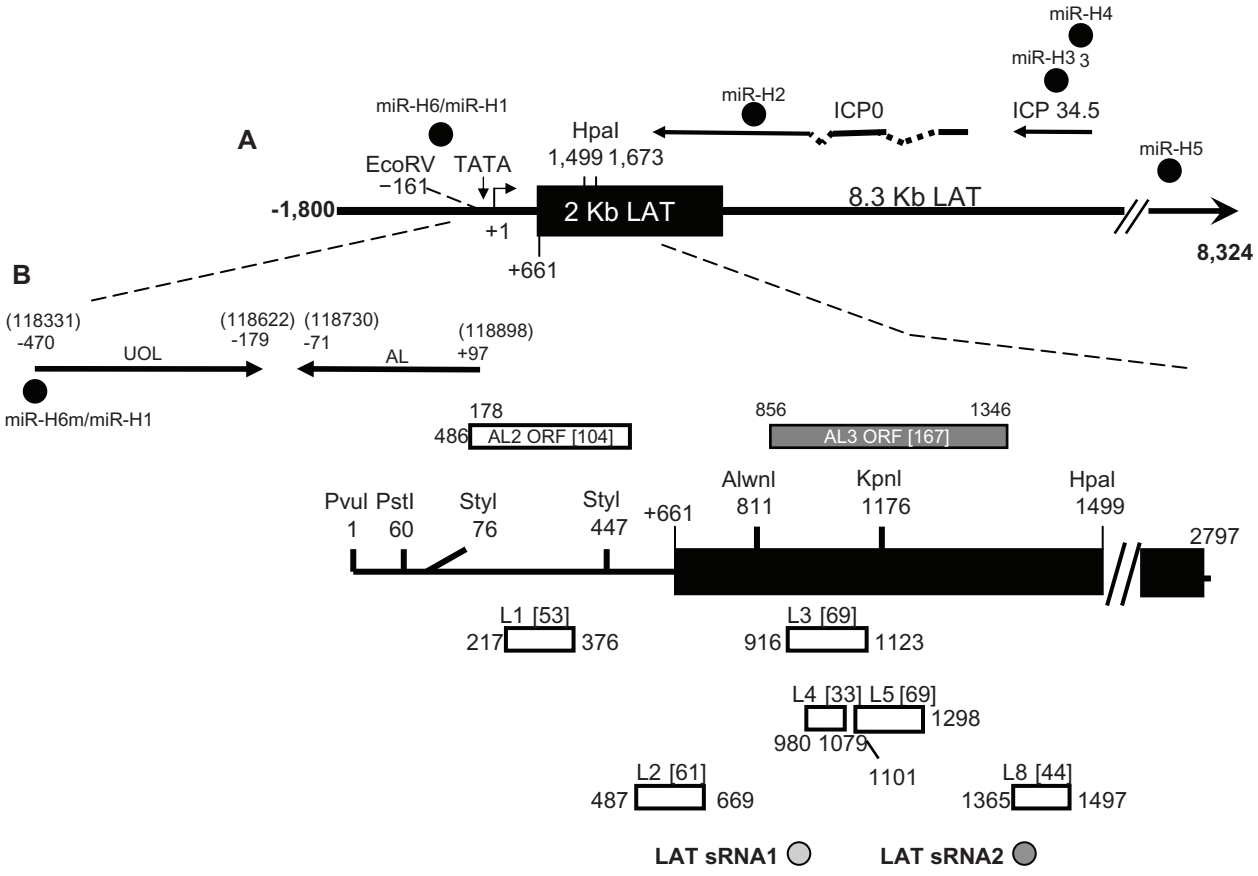

Figure 2. Schematic of putative factors encoded within the LAT locus. (Panel **A**) Schematic of genes within the long repeats that contain the LAT locus. The large arrow indicates the primary LAT transcript. The solid rectangle represents the sTable 2 kb LAT intron. Initiation of LAT transcription is denoted by the arrow at +1 (genomic nucleotide 118801). Several restriction enzyme sites and the relative locations of the ICP0 and ICP34.5 transcripts are shown for reference. The location of the 6 microRNAs (miR-H1-6) that is located within the 8.5 kb LAT[128] are shown. (Panel **B**) Partial restriction map of LAT and position of LAT open reading frames (L1-8) within the first 1.5 Kb of strain McKrae LAT coding sequences, which were based on previous studies.[148] The numbering system of the ORFs was consistent with a previous study.[148] Only the ORFs with at least 30 amino acids are shown (the number of amino acids in each ORF is denoted by the numbers in brackets). Open circles denote the position of two LAT small RNAs that are encoded within the first 1.5 kb LAT coding sequences.[258] Positions of UOL transcript, AL transcript, and ORFs located on the opposite strand of LAT (AL2 and AL3) are shown. The number of amino acids of AL2 and AL3 are in brackets. Nucleotide positions relative to the start of LAT transcription are not shown in parenthesis.

studies provide evidence that the LAT encoded miRNAs and sRNAs promote latency by interfering with expression of important viral transcriptional regulatory proteins.

The LAT locus encodes additional transcripts (Fig. 2B). More than one transcript, including UOL (Upstream of LAT)[131] are located within LAT promoter sequences. Expression of the UOL transcript or protein does not reduce reactivation from latency in rabbits.[132] An antisense to LAT (AL) transcript is expressed from the first 1.5 kb of LAT coding sequences and appears to encode a protein.[133] Two additional small open reading frames (ORFs) that are antisense to LAT (AL2 and AL3) are present in LAT coding sequences. An AL3 transcript is expressed during productive infection and in TG of mice latently infected with wild-type HSV-1, but not a LAT null mutant virus.[134] An AL3-specific polyclonal antibody detected a protein in a subset of TG neurons in latently infected mice. A transcript encompassing AL2 has not been detected during productive infection or latency (unpublished data).

LAT null HSV-1 mutants have been examined in various small animal models.[2,3] Although two studies concluded that LAT does not play a role in latency,[135,136] most have provided evidence that LAT is important. This discrepancy may be due to the strain of virus or mouse that was used for specific studies. LAT enhances the establishment of latency in mice[137,138] and in rabbit ocular infection models,[139] in part by reducing lytic cycle viral gene expression in TG of mice.[140,141] By enhancing the establishment of latency, LAT would increase the pool of latently infected neurons; thus indirectly increasing the incidence of reactivation from latency.

As a result of stress or other external stimuli, reactivation from latency can occur, resulting in virus shedding (Fig. 4). The McKrae strain of wild-type HSV-1, however not a LAT null mutant, is consistently detected in tears of infected rabbits, due to spontaneous reactivation.[139,142–145] These same LAT null mutants grow with wild-type efficiency in cultured cells and in acutely infected rabbits. When just the first 1.5 kb of LAT coding sequences (Fig. 2B) is expressed from the HSV-1 genome, wild-type levels of spontaneous reactivation from latency occur in rabbits.[139] Similar results were observed using another virulent strain of HSV-1 (17 syn+) in a rabbit eye model.[146,147] The first 1.5 kb of LAT coding sequences does not overlap ICP0, suggesting that antisense repression of ICP0 expression by LAT is not important for spontaneous reactivation in the rabbit ocular model of infection. The factors encoded by the first 1.5 kb of LAT coding sequences that promote spontaneous reactivation have not been fully characterized.

Although certain studies suggested LAT does not encode a protein,[148] other studies have concluded that a protein encoded within LAT sequences is expressed.[131,149–154] These proteins were suggested to either substitute for ICP0 functions,[153,154] interfere with binding of ICP4 to DNA,[152] or their functions were not described. The proposed LAT proteins are mapped downstream of the critical first 1.5 kb of the primary LAT transcript, a region that appears both sufficient and necessary for the wild type spontaneous reactivation phenotype in rabbit models.[139,155] Within the first 1.5 kb of LAT coding sequences, 8 potential ORFs have been identified in the McKrae strain (Fig. 2B).[148] The L2 ORF (Fig. 2B) appears to be expressed in TG of latently infected mice.[156] Although LAT is not absolutely required for the latency-reactivation cycle in small animal models, its importance may be underestimated using small animal models and measuring latency in terms of weeks or months, not decades.

The BHV-1 latency related RNA is abundantly expressed in sensory neurons and is necessary for reactivation from latency

Latency related (LR) RNA is abundantly expressed in TG neurons of calves that are latently infected.[111,157] Two different start sites of LR-RNA transcription (Fig. 3A) have been identifed suggesting this has functional significane. It is clear that the LR gene encodes more than one product.[11,36] For example, the LR gene contains two-well defined ORFs (ORF2 and ORF1; Fig. 3B) 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 (Fig. 3A),[158,159] ORF2 can be fused with ORF1 protein coding sequences or RF-B. The ORF2/ORF1 fusion protein stably interacts with the cellular transcription factor C/EBP-alpha.[160] C/EBP-alpha RNA and protein levels increase in TG neurons

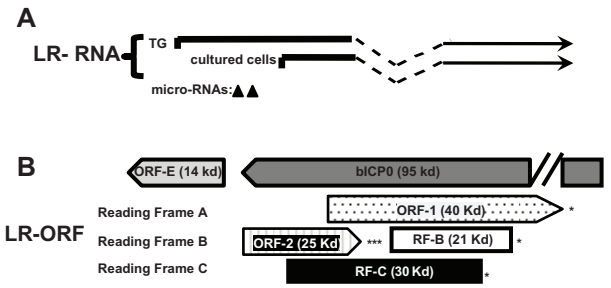

Figure 3. Schematic of the BHV-1 LR gene and surrounding genes. (Panel **A**) The start sites for LR transcription during latency and productive infection were previously described.[159,259] (Panel **B**) Organization of LR ORFs and 3′ terminus of bICP0. ORF-1 and ORF-2 are located in the LR gene and have the potential to encode a 40 or 25 kd protein respectively. **Notes:** Reading Frames B (RF-B) and C (RF-C) are open reading frames that lack an initiating Met. The (*) denotes 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.

during dexamethasone induced reactivation from latency. Over-expression of C/EBP-alpha enhanced productive infection,[161] suggesting that ORF2 sequesters C/EBP-alpha and reduces the efficieny of productive infection during the latency-reactivation cycle.

One day after calves are infected and during latency, splicing of LR-RNA in TG is such that ORF2 is intact,[159] suggesting ORF2 expression is important for the latency-reactivation cycle. ORF2 interacts with Notch1 and Notch3, components of the Notch signaling pathway.[162] Mammalian Notch receptor family members (Notch1-4) are membrane tethered transcription factors that regulate many developmental and physiological processes.[163,164] For example, Notch promotes neuronal maintenance, development, and differentiation.[165–167] Notch3[168] and Notch1[169,170] promote cell survival by activating a protein kinase, (AKT) which inhibits apoptosis. Notch family members can also induce apoptosis,[163,164] suggesting Notch influences cell survival by cell-type dependent mechanisms. 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 nucelus, Notch ICD interacts with members of the CSL family of transcriptional factors, CBF1, Su(H), or Lag1 (also referred to as RBP-J binding proteins) subsequently activating downstream genes. Notch1, but not Notch3, enhances BHV-1 productive infection[163] and Notch1 activates the BHV-1 immediate-early transcription unit 1 (IEtu1) and bICP0 early promoters. Notch1 and Notch3 trans-activated the late glycoprotein C (gC)

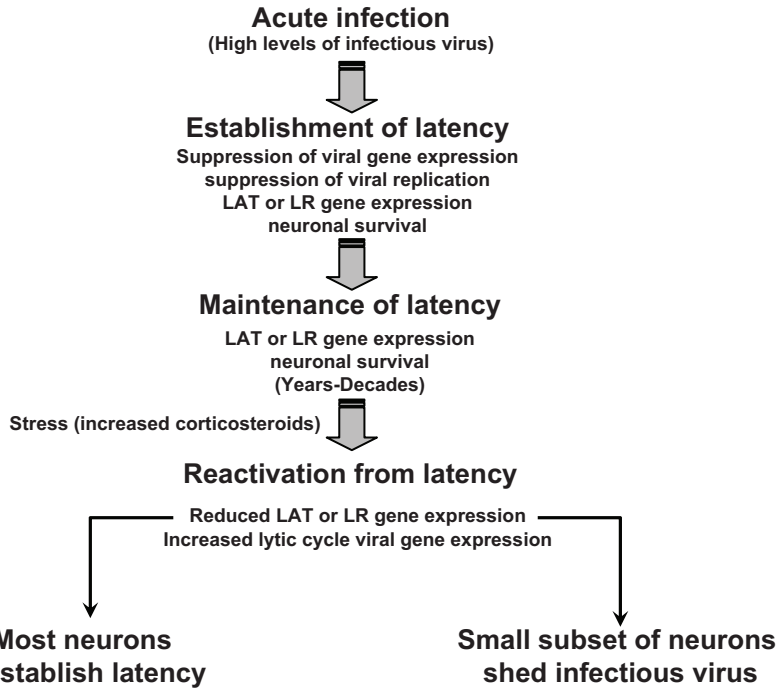

Figure 4. Putative steps that occur during the latency-reactivation cycle.
Note: For details, see text.

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[162] suggesting this function is important for establishing and/or maintaining latency. Notch3 RNA levels are higher during dexamethasone (DEX) induced reactivation from latency, suggesting Notch family members stimulate productive infection during reactivation from latency. Activation of Notch signaling in post-mitotic-neurons or neuroblastoma cells inhibits neurite sprouting[165,171–174] and axon repair,[175] which can lead to neuronal degeneration and apoptosis.[176–178] Conversely, neurite sprouting correlates with regeneration of damaged axons and dendrites.[175] ORF2 promotes neuruite sprouting and neuronal differentiation of mouse neuroblastoma cells when Notch1 or Notch3 is over-expressed.[179] Collectively, these studies suggest that ORF2 interactions with Notch family members promote the establishment and maintenace of latency by (1) interfering with viral gene expression necessary for productive infection, (2) supporting a mature neuronal phenotype, and (3) overcoming the deleterious effects of Notch expression during stress-induced reactivation from latency.

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.[180,181] 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 wild-type LR gene, suggesting expression of a LR protein is not required.[181] LR gene products also inhibit mammalian cell growth,[182,183] and the cell growth inhibitory function of the LR gene maps to a 463-bp fragment that lacks a significant open reading frame.[182] Two miRNAs located upstream of ORF2 are expressed during latency.[184] These miRNAs, or larger sRNAs containing these miRNAs, reduced bICP0 protein levels in transient transfection assays.

A small ORF located within the LR promoter is designated ORF-E (Fig. 3B). ORF-E is antisense to the LR transcript and is downstream of bICP0 coding sequences, but does not overlap bICP0. A transcript that encompasses ORF-E is expressed during productive infection and in TG of latently infected calves.[185] The LR promoter contains multiple cis-acting motifs, has a neuronal specific binding domain,[186–188] and contains a long AT-rich motif (40/53 nucleotides are A or T) that may promote ORF-E transcription. 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,[189] which may support a mature neuronal phenotype following infection.

LAT and LR Gene Products Inhibit Apoptosis

LAT inhibits apoptosis

LAT expressing plasmids interfere with apoptosis in transiently transfected cells, and LAT expressing viruses inhibit apoptosis in TG of infected mice or rabbits.[117,190–192] The anti-apoptotic functions of LAT correlate with promoting spontaneous reactivation from latency.[191,193] In the context of promoting spontaneous reactivation from latency in the rabbit model (model), inhibiting apoptosis is the most important function of LAT as three different anti-apoptosis genes[129,194–196] restore wild-type levels of spontaneous reactivation from latency to a LAT null mutant. LAT may encode other functions because the LAT null mutants that express cellular anti-apoptosis genes have reduced virulence, in spite of reactivating from latency with wild-type frequency. LAT expressing plasmids, in the absence of other viral genes, inhibit caspase 8- and caspase 9-induced apoptosis,[193,197] the two major apoptotic pathways in mammals.[198–200] LAT also inhibits caspase 3 activation.[201]

LAT sRNA1 and sRNA2 cooperate to inhibit cold-shock induced apoptosis in mouse neuroblastoma cells.[130] Introduction of ATG→TTG mutations in ORFs within the first 1.5 kb of LAT coding sequences impairs the anti-apoptotic functions of LAT,[202] suggesting that LAT either encodes a functional protein or alters RNA structure. Two of these ATG→TTG mutations are within LAT sRNA1 and sRNA2, and introducing these mutations into the small RNAs inhibits

their ability to inhibit apoptosis.[130] At this time, it is not clear how these sRNAs interfere with apoptosis. It will also be important to construct a recombinant virus with these same mutations and test whether the spontaneous reactivation incidence is affected.

LAT also inhibits GrzB induced apoptosis in transient transfection studies.[203] GrzB is released from $CD8^+$ T cells as well as other specific lymphocytes; GrzB has features similar to apical caspases, and can induce apoptosis in most cell types.[204–207] Inhibiting GrzB induced apoptosis may be important for the latency-reactivation cycle because $CD8^+$ T lymphocytes control HSV infection in sensory ganglia.[208,209]

The LR gene encodes more than one product that inhibits apoptosis

A mutant BHV-1 strain with 3 stop codons after the initiating methionine codon of ORF-2 (LR mutant virus) does not express detectable levels of ORF-2[101] but expresses reduced levels of ORF1 in cultured cells during productive infection.[210] The LR mutant virus grows less efficiently in the ocular cavity and TG, but grows almost as efficiently as wild-type BHV-1 in the nasal cavity, and does not reactivate from latency following DEX treatment.[211,212] The LR mutant virus induces higher levels of apoptosis in TG neurons of infected calves,[213] and a LR gene expressing plasmid with the same stop codon mutations does not effectively inhibit apoptosis.[214,215] ORF2 expression in the absence of other viral genes inhibits apoptosis in transiently transfected cells,[216,217] suggesting that ORF2 is a dominant function encoded by the LR gene. ORF2, like LAT, can inhibit caspase 8 and caspase 9 mediated apoptosis; however the mechanism by which it inhibits apoptosis is not known.

Two microRNAs encoded within the LR gene (Fig. 3A) interfere with bICP0 protein expression[184] and cold shock induced apoptosis in transfected mouse neuroblastoma cells (Neuro-2A cells).[218] Since cold shock induced apoptosis in Neuro-2A cells is inhibited by casapse 3 and caspase 9 inhibitors,[219] the microRNAs must influence these apoptotic signaling pathways. The ability of the microRNAs to stimulate the anti-apoptotic transcription factor NF-αB[220–223] seems to be important for inhibiting cold-shock induced apoptosis. In summary, these results provide additional evidence that interfering with apoptosis is crucial for a successful life-long latent infection.

Why is Inhibiting Neuronal Apoptosis Important During the Latency-Reactivation Cycle?

The latency-reactivation cycle has been operationally divided into three distinct steps: establishment, maintenance, and reactivation (Fig. 4). Following acute infection where high levels of infectious virus are produced, virus particles enter sensory neurons. Initial entry of the viral genome into a sensory neuron results in a burst of lytic cycle viral gene expression and infectious viruses are produced. Viral gene expression is then extinguished, with the exception of HSV-1 LAT and BHV-1 LR gene products. Neuronal cell factors,[2,9] LAT encoded microRNAs plus sRNAs,[128,130] and LR encoded functions[162,184] interfere with various aspects of productive infection. During acute infection and establishment of latency, neuronal and satellite cells undergo apoptosis when small animal models are infected with HSV-2[224] or HSV-1.[225–227] BHV-1 replication and gene expression also occur in TG of acutely infected calves, resulting in apoptosis of neurons and non-neuronal cells.[192,213,228]

HSV-1 LAT[192] and the LR gene[213] enhance neuronal survival during the establishment of latency. The ability of LAT[229] and the LR gene[179] to promote a mature neuronal phenotype and sprout neurites may also promote establishment of latency by stimulating repair of damaged neurons following infection. Successful establishment correlates with an increase in the number of infected neurons that survive and enhances the probability that reactivation from latency occurs.

Maintenance of latency is a phase that lasts for the duration of the host's life and is operationally defined as a period when infectious virus is not readily detected. In general, abundant expression of viral genes required for productive infection does not occur. LAT or LR gene products are abundantly expressed during the maintenance of latency. Expression of LAT correlates with an increase of latently infected neurons during the maintenance of latency,[230] suggesting latently infected sensory neurons are exposed to apoptotic stimuli during the maintenance of latency. It is reasonable to predict that LAT and LR gene products

actively participate in maintaining a latent infection in sensory neurons.

Reactivation from latency is initiated by external stimuli (stress, immunosuppression, or UV light for example), which ultimately must stimulate viral gene expression.[36,231,232] Abundant viral gene expression can be detected in sensory neurons and infectious virus can be isolated from TG, ocular swabs, and/or nasal swabs. Stress leads to elevated corticosteriod levels, which has rapid effects on neural activity.[233,234] DEX, a synthetic corticosteriod, induces viral gene expression,[235] stimulates an HSV-1 origin of replication (Ori-L) in neuronal cells,[55] and alters splicing patterns in the absence of protein synthesis.[236] DEX and other apoptosis stimulators can also stimulate HSV-1 reactivation from latency.[237,238] BHV-1 reactivation from latency is induced by DEX, in part because it stimulates expression of cellular transcription factors and viral gene expression while repressing expression of LR gene products.[9,11,36] Prolonged exposure to corticosteroids can also induce immunosuppression, in part by inducing apoptosis in lymphocytes.[239] A subset of neurons that successfully reactivate from latency to produce infectious virus may not survive;[240–242] however it is not clear if this is the fate for all neurons that produce infectious virus during reactivation from latency. Most latently infected neurons that are exposed to reactivation stimuli re-establish latency and do not produce infectious virus.[243,244] Given that sensory neurons are terminally differentiated cells, inhibiting apoptosis during the latency-reactivation cycle is crucial for life-long latent infections of α-herpesvirinae subfamily members.

Numerous studies have demonstrated that infiltrating lymphocytes in TG regulate the latency-reactivation cycle. For example, a persistent cell-mediated immune response occurs in TG during latency and $CD8^+$ T lymphocytes inhibit reactivation from latency.[208,209,245–250] Release of granzyme B from CD8+ T cells into latently infected neurons helps to inhibit reactivation from latency by cleaving the viral transcriptional trans-activator, ICP4.[251] Since it is well established that granzyme B activates caspase 3 and the intrinsic pathway of apoptosis,[207] the ability of LAT and perhaps LR gene products to inhibit apoptosis is important to overcome the effects of granzyme B. The ability of HSV-1 to inhibit major histocompatibility complex (MHC) class I presentation in sensory neurons correlates with successful reactivation[252] providing further evidence that $CD8^+$ T cells monitor latently infected neurons. In conclusion, the ability of HSV-1, HSV-2, and BHV-1 to reactivate from latency is regulated by complex virus-host interactions.

Perspectives

Although genetic and functional studies have demonstrated that LAT and the LR gene regulate the latency-reactivation cycle, there are many unanswered questions. For example, identifying the functions of various transcripts, the small non-coding RNAs, and the ORFs within LAT are crucial to understand the role these various factors play in the latency-reactivation cycle. It would not be surprising to find that one or more of these factors encoded within the LAT locus regulate certain neuronal specific functions that maintain normal functions. It will be difficult to make additional LAT mutant viruses as many of these factors overlap and deletion of these sequences would likely interfere with expression of more than one LAT encoded factor. Consequently, many of these studies will have to be performed in transient transfection assays in primary neurons or neuroblastoma cells. Finally, examining LAT in small animal models in terms of weeks after acute infection may not accurately reflect the latency-reactivation cycle in the context of life-long latency in humans.

With respect to the LR gene, there are no studies that have determined whether ORF1, ORF-E, or the microRNAs play a role in the latency-reactivation cycle. Furthermore, functional analysis of ORF-1 and ORF-E has not been performed. Identifying the cellular proteins that interact with ORF-1 and ORF-E may provide insight into their functions. In summary, the finding that sequences encompassing LAT and the LR gene encode for more than one transcript and/or small non-coding RNAs implies many functions are necessary to successfully regulate the life-long latency-reactivation in the natural host.

Author Contributions

Conceived and designed the experiments: CJ. Analyzed the data: CJ. Wrote the first draft of the manuscript: CJ. Contributed to the writing of the manuscript: CJ. Agree with manuscript results and conclusions: CJ. Jointly developed the structure and arguments for the paper: CJ. Made critical revisions and approved final version: CJ. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the author has provided signed confirmation of compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests. Provenance: the authors were invited to submit this paper.

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