

Review Article

Towards an Understanding of the Herpes Simplex Virus Type 1 Latency-Reactivation Cycle

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Infection by herpes simplex virus type 1 (HSV-1) can cause clinical symptoms in the peripheral and central nervous system. Recurrent ocular shedding can lead to corneal scarring and vision loss making HSV-1 a leading cause of corneal blindness due to an infectious agent. The primary site of HSV-1 latency is sensory neurons within trigeminal ganglia. Periodically, reactivation from latency occurs resulting in virus transmission and recurrent disease. During latency, the latency-associated transcript (LAT) is abundantly expressed. LAT expression is important for the latency-reactivation cycle in animal models, in part, because it inhibits apoptosis, viral gene expression, and productive infection. A novel transcript within LAT coding sequences (AL3) and small nonprotein coding RNAs are also expressed in trigeminal ganglia of latently infected mice. In this review, an update of viral factors that are expressed during latency and their potential roles in regulating the latency-reactivation cycle is discussed.

1. Introduction

At least 90% of the population are infected with herpes simplex virus type 1 (HSV-1), and infection can cause a variety of disorders [1, 2]. Recurrent ocular HSV-1 is the leading cause of infectious corneal blindness in industrialized nations [3]. In a murine model, ocular infection appears to induce autoimmune disorders leading to corneal antigen destruction and stromal keratitis [4]. HSV-1 infections also cause gastrointestinal disorders, esophageal disorders, and approximately 25% of all genital herpes infections [5, 6].

HSV-1 is the most commonly identified cause of acute, sporadic viral encephalitis in the U.S. accounting for 10%–20% of all cases [7]. It is estimated that there are approximately 2,000 new cases per year in the U.S. HSV-1 and HSV-2 can cause acute necrotizing encephalitis in infants, children, and adults. Encephalitis due to HSV-2 in newborn infants is a widespread disease in the brain and commonly involves a variety of other organs in the body including skin, eyes, and lungs [8].

Herpes simplex virus-induced encephalitis (HSE) is characterized by severe destruction of temporal and frontal lobe structures, including limbic mesocortices, amygdala, and hippocampus. Without antiviral therapy, the mortality rate is as high as 70%, but even after antiviral therapy 20% of these patients die. Despite early treatment, chronic progressive tissue damage in magnetic resonance imaging can be found up to 6 months following the onset of symptoms. Approximately 2/3 of the HSE cases occur because of reactivation from latency [9], which explains why there is high morbidity and long-term complications despite antiviral treatment [10–12].

HSE is often associated with necrotic cell death resulting from virus replication and inflammatory changes secondary to virus-induced immune response [13]. However, there is not a perfect correlation between virus burden in the brain and the severity of histological changes and neurological symptoms. Furthermore, a small number of HSE patients are negative for HSV-1 DNA early in the course of infection suggesting that factors other than virus replication are

involved in pathogenesis. The finding that mice lacking toll like receptor 2 (TLR2) are less susceptible to HSV-1-induced encephalitis than wild type mice or mice lacking TLR4 implies that TLR-2-mediated cytokine responses are detrimental to the host [14]. In addition, two children with HSE lack the intracellular protein UNC-93B and have impaired interferon responses [15]. It appears that the ability of the host to appropriately respond to HSV-1 infections in the brain is crucial for preventing HSE.

2. The Latency-Reactivation Cycle

2.1. The Latency-Reactivation Cycle Has 3 Distinct Steps. Despite a vigorous immune response during acute infection, HSV-1 establishes latency in ganglionic sensory neurons, typically trigeminal ganglia (TG) or sacral dorsal root ganglia [16, 17]. Although TG is a primary site of latency following ocular, oral, or intranasal infection [18–20], latent HSV-1 can also be detected in human adult nodose ganglia and the vagus nerve [5, 6]. Up to 40% of sensory neurons can be latently infected [21–25]. HSV-1 genomic DNA has also been detected in the central nervous system of a significant percentage of humans [18, 26, 27].

The steps of the latency-reactivation cycle have been operationally divided into three major steps: establishment, maintenance, and reactivation (Figure 1). Establishment of latency includes entry of the viral genome into a sensory neuron and acute infection. Viral gene expression is then extinguished, with the exception of the latency-associated transcript (LAT). For further details regarding viral gene expression during acute infection and establishment of latency, see Section 2.2.

Maintenance of latency is a phase that lasts for the life of the host and is operationally defined as a period when infectious virus is not detected by standard virus isolation procedures. In general, abundant expression of viral genes that are required for productive infection does not occur. LAT is abundantly expressed during this stage of latency.

Reactivation from latency is initiated by external stimuli (stress and immunosuppression, e.g.) that stimulate viral gene expression. Abundant viral gene expression is detected in sensory neurons and infectious virus can be isolated from TG, eye swabs, and/or nasal swabs. It is not clear whether a neuron that undergoes reactivation and produces infectious virus survives and resumes latency or is killed. For further discussion of factors that regulate reactivation from latency, see Sections 2.4–2.6. The ability of HSV-1 to reactivate from latency results in recurrent disease and virus transmission.

2.2. Viral Gene Expression during

Productive Infection versus Latency

2.2.1. Viral Gene Expression during Productive Infection. Binding and entry of HSV-1 to cells are mediated by viral glycoproteins and cellular factors [28–30]. A cellular mediator of viral entry (HveA or HVEM) is primarily expressed in activated T cells and belongs to the tumor necrosis factor receptor family [31]. Entry of HSV-1 into

epithelial and other nonlymphoid cells is mediated by an unrelated membrane glycoprotein that resembles the poliovirus receptor (HveB and HveC) [32]. HveC is active as an entry mediator for all herpesviruses examined to date, HSV-1, bovine herpesvirus 1 {BHV-1}, and pseudorabies virus, {PRV}. HveC is abundantly expressed in neurons and can block viral entry in several neuron-like cell lines [32]. After uncoating, the viral genome is present in the nucleus and viral gene expression ensues.

HSV gene expression is temporally regulated in three distinct phases: immediate early (IE), early (E), or late (L) [33]. IE transcription does not require protein synthesis and is stimulated by VP16 [34]. E gene expression is dependent on at least one IE protein, and generally E genes encode nonstructural proteins that play a role in viral DNA synthesis. L gene expression is maximal after viral DNA replication, requires IE protein production, and L proteins comprise the virion particle.

Five IE genes encode ICP0, ICP4, ICP22, ICP27, or ICP47. ICP4 [35–38] and ICP27 [39–41] are required for virus growth in tissue culture. In general, ICP4 represses IE gene expression [37, 42–46] and activates E or L gene expression by interacting with RNA polymerase II transcription factors [46, 47]. ICP27 redistributes small nuclear ribonucleoprotein complexes, interferes with splicing of IE transcripts, and promotes E and L poly A site selection [48–51]. ICP47 prevents transport of antigenic peptides into the endoplasmic reticulum [52] and is crucial for neurovirulence because it inhibits CD8⁺ T cell responses [53]. ICP0 can activate expression of all classes of viral genes, in part because it increases steady-state levels of mRNA [54].

ICP0 also binds several cellular proteins: (1) elongation factor 1 α [55], (2) cyclin D3 [56], (3) an ubiquitin-specific protease [57, 58], and (4) PML [59–62]. Interactions between ICP0 and chromatin-remodeling enzymes activate viral transcription. For example, a histone deacetylase inhibitor enhance viral gene expression and productive infection [63, 64]. Secondly, ICP0 alters a complex that inhibits gene expression (REST/CoREST/histone deacetylase repressor complex) [65]. Finally, HSV-1 ICP0 interacts with HDAC2 [66] and blocks histone deacetylation to stimulate viral gene expression [65, 67]. Since ICP0 has recently been shown to remove histones from viral chromatin during productive infection [68], it is tempting to suggest that ICP0 has a similar function during reactivation from latency. These activities of ICP0 promote virus replication in differentiated cells [69].

2.2.2. Viral Gene Expression Is Extinguished after Infection of Sensory Neurons.

Following infection of rodents, rabbits, or humans with HSV-1, productive infection is initiated in the mucosal epithelium. Virus particles or subparticles then enter sensory neurons and are transported intra-axonally to the sensory ganglia. Since HSV-1 infection typically occurs via the oral, ocular, or nasal route, the 5th cranial nerve, trigeminal ganglia (TG), is a primary site for latency [18, 19]. Extensive viral gene expression and replication occur within TG for approximately a week following infection of animal

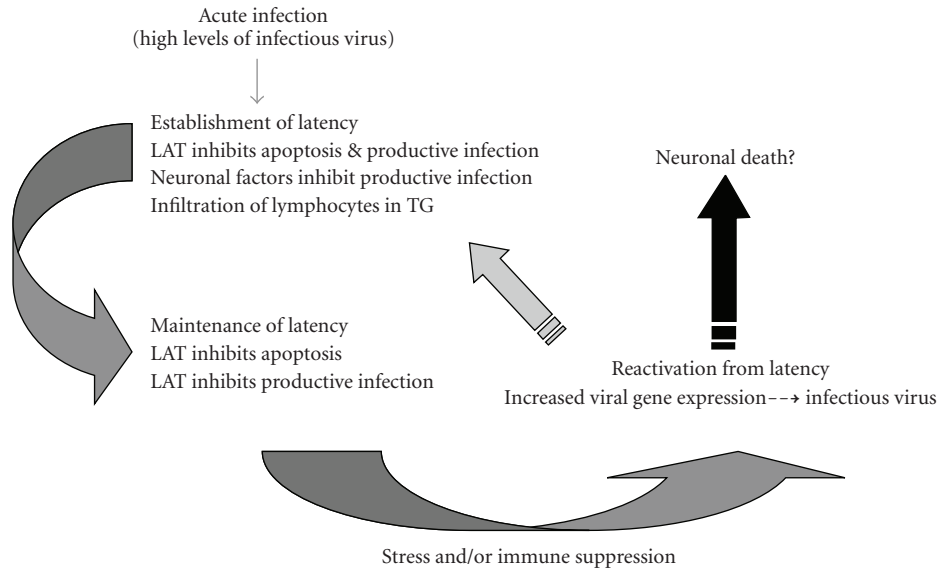


FIGURE 1: Steps in the latency-reactivation cycle of HSV-1. For details, see the text.

models that support HSV infection [70, 71]. Productive viral gene expression that occurs in TG appears to be different than what is seen in cultured cells [72]. Infectious virus can readily be detected in homogenates prepared from TG during acute infection. However, it is difficult to conclude whether this infectious virus is the result of productive infection in sensory neurons or the result of transport from peripheral sites of infection. Replication is not required for establishment of latency because mutants that cannot replicate will establish latency, but at a reduced level [73–81].

2.2.3. IE Promoters Are Differentially Regulated in Sensory Neurons Relative to Nonneuronal Cell Types. Several studies using transgenic mice that contain IE promoters linked to a reporter gene have concluded that IE promoters are differentially regulated by neuronal specific factors. For example, the HSV-1 ICP4 promoter is active in Schwann cells, but not sensory neurons in TG [82]. As expected, the ICP4 promoter in transgenic mice is activated in TG neurons following infection with HSV-1. In contrast to the ICP4 promoter, transgenic mice containing the ICP0 or ICP27 promoters are active in certain neurons within the brain and TG [83]. The ICP0 promoter is also differentially regulated in TG neurons depending on the age of the mouse. The ICP0 promoter contains a cis-acting element that can bind a neuronal specific transcription factor, Olf-1, which is differentially and developmentally expressed in specific subsets of sensory neurons [84] suggesting that the Olf-1 site plays a role in activating ICP0 promoter activity in certain neurons.

All IE promoters contain a common cis-acting sequence (TAATGARAT) that is required for VP16-mediated transactivation [34, 85]. VP16 must interact with two cellular proteins, Oct-1 and HCF, to efficiently induce IE promoter activity. A cellular transcription factor, Zhangfei, binds to

HCF and prevents activation of the ICP0 promoter [86]. Another cellular transcription factor, Luman, also binds to HCF and sequesters HCF in the cytoplasm of sensory neurons, suggesting that Luman has a role in latency [86]. Zhangfei and Luman have basic domain-leucine zippers (bZIP) regions, acidic activation domains, and consensus HCF-binding motifs, yet have little amino acid similarity. In nonneuronal cells, HCF has a nuclear localization [87], but in sensory neurons it appears to be predominantly localized to the cytoplasm [88]. If the relative levels of Luman and Zhangfei are high, the availability of “free” HCF that could interact with VP16 would be reduced and consequently IE gene expression would be repressed. It has also been hypothesized that VP16 is not present in sufficient quantities in the nucleus of infected sensory neurons to stimulate efficient productive infection [88]. However, inducible expression of VP16 in the context of the viral genome or in transgenic mice did not lead to enhanced viral replication [89].

Other cellular transcription factors expressed in sensory neurons (Brn-3.0 and N-Oct3, e.g.) have the potential to regulate IE gene expression [90, 91]. Brn-3.0 binds to noncoding sequences in the HSV-1 genome, but the binding sites for Brn-3.0 are not identical to those for Oct-1 or other related transcription factors that also include Brn-3.1 and Brn-3.2 [92]. Brn-3.0 is important in the peripheral nervous system of mice because null mutations in the *brn-3.0* locus result in neonatal death with defects in sensory ganglia and specific central nervous system nuclei [93, 94]. *brn-3.2* is required for differentiation of certain retinal ganglion cells [95]. One study has concluded that Brn-3.1 and 3.2 have opposite effects on a target promoter [96]. Considering that the Brn3 family of transcription factors is expressed in the peripheral nervous system, these proteins may regulate HSV gene expression during the latency-reactivation cycle.

Following infection of primary neurons, ICP0 does not appear to accumulate in the nucleus of infected cells [97]. An independent study also concluded that the function of ICP0 is impaired in human neuronal-like cells because a nuclear structure (ND10) that ICP0 interacts with is different compared to nonneuronal cells [98]. The same neuronal-like cells do not support efficient viral replication, in part, because ICP0 expressing plasmids do not activate viral transcription efficiently. These studies argue that ICP0 does not function efficiently in neuronal cells and thus productive infection is inhibited.

2.2.4. The LAT Promoter Is Neuronal Specific. In sharp contrast to other HSV-1 promoters, the promoter that directs expression of the latency-associated transcript (LAT) is activated in sensory neurons (see Figure 2 for a schematic of the HSV-1 LAT promoter). Two separate promoter fragments that are upstream of the start site of LAT, latency-associated promoter 1 and 2 (LAP1 and LAP2), can cis-activate a reporter gene in transiently transfected cells [99, 100]. Several studies have demonstrated that sequences spanning the TATA box, LAP1, are critical for directing LAT expression in sensory neurons [99, 101–104]. LAP2 promoter has been proposed to promote expression of the stable 2 Kb LAT expression during productive infection of cultured cells. LAP2 may also play a role in promoting long-term expression of LAT in sensory neurons or may activate expression of novel transcripts during specific stages of infection in sensory neurons. Although the LAT promoter elements have neuronal specificity in transient transfection assays, they can also direct expression of a reporter gene in nonneural cells [105–109]. This may reflect the abundance of cellular transcription factor binding sites within the LAT promoter (Figure 2(c)). Many of these transcription factors are present in nonneural cells and can activate the LAT promoter in transiently transfected cells. For example, the two CRE binding sites in the LAT promoter are functional because cAMP activates the promoter [110, 111]. The CRE motif that is proximal to the TATA box is important for expression in neurons, and its presence has a positive effect on reactivation from latency [111–113]. Furthermore, Sp1, YY1, USF, and CAAT are frequently found in RNA polymerase II promoters that are not neural specific. Neuronal specific factors have been identified that bind to the LAT promoter [111–113]. The finding that the IE protein, ICP4, binds to DNA sequences downstream of the TATA box and represses the LAT promoter is one important reason why LAT is not an abundant transcript during productive infection [107].

Long-term expression of LAT has also been examined in the context of the viral genome [122–125]. These studies have demonstrated that LAP2 sequences function as a long-term enhancer (Figure 2(c)) in latently infected mice. LAP2 also appears to maintain LAP1 promoter activity. Although DNA sequences within the LAT promoter activate RNA expression in sensory neurons, neuronal specificity does not appear to be contained into a single cis-acting motif. As expected, the LAT locus is transcriptionally active during latency and is associated with acetylated histones, whereas ICP0 expression is repressed and hypoacetylated [126].

2.3. *Viral Gene Expression Is Restricted during Latency to the LAT Locus*

2.3.1. LAT Is Abundantly Expressed in Sensory Neurons during Latency. LAT is abundantly transcribed in latently infected neurons of mice, rabbits, or humans [1, 104, 114, 115, 127–131]. Mice, rabbits, or humans latently infected with HSV-1 express LAT, and LAT is predominantly detected in the nucleus. LAT is complementary to ICP0 and overlaps the ICP0 transcript (Figure 2(b)), suggesting that 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 do not overlap ICP0 [132]. The simplest interpretations of these data are that LAT has more than one function or the ability of LAT to repress ICP0 expression is not that important in the small animal models used to study latency.

Detection of thymidine kinase and ICP4 transcripts, in addition to LAT, in TG of latently infected mice [133] appears to be the result of spontaneous reactivation or unsuccessful reactivation from latency [134, 135]. Viral genome positive neurons that are LAT negative can be detected in latently infected mice [24]. Since *in situ* PCR was used to detect viral DNA, but *in situ* hybridization was used to detect LAT, neurons expressing low levels of LAT were likely missed.

Splicing of the 8.5 Kb LAT transcript yields an abundant 2 Kb LAT and an unstable 6.5 Kb LAT [109, 114, 128] (Figure 3). Correct splicing of the 2 Kb LAT is necessary for establishment and maintenance of latency [139, 140]. In general, the stable 2 Kb LAT is not capped, is poly A-appears to be circular, and is a stable intron [141, 142]. Although LAT is predominantly detected in the nucleus, it is also present in the cytoplasm [143–145] and is associated with polyribosomes [143, 146] or splicing factors [143].

2.3.2. Small Nonprotein Coding RNAs Are Encoded within the LAT Locus. Small noncoding RNAs can regulate gene expression [147, 148], promote neuronal differentiation [149], or inhibit apoptosis [150]. There are numerous types of small noncoding RNA: short interfering (si) RNA [151], small temporal RNA [152], heterochromatic siRNA [153], tiny noncoding RNAs [154], and micro-RNAs (miRNAs) [155]. miRNAs are nonprotein coding RNA molecules that are synthesized in the nucleus as 70–90 nucleotide precursors, and then processed into 21–23 nucleotide single-stranded RNA by the Dicer nuclease in the cytoplasm [155]. Dicer also processes siRNA. Following the discovery of 5 miRNAs encoded within the Epstein-Barr virus genome [156], miRNAs have been identified in Kaposi sarcoma-associated virus [157–159], mouse gammaherpesvirus 68 [157], human cytomegalovirus [157, 160, 161], HSV-1 [156, 162], Marek's disease virus [163], and simian virus (SV40) [164].

A study by Umbach et al. [136] concluded LAT is a miRNA precursor that encodes four miRNAs, and two within LAT promoter sequences (Figure 3(a)). One of these miRNAs, LAT miR-H6, inhibits ICP4 protein levels but not ICP4 RNA levels. ICP0 protein levels, but not RNA levels,

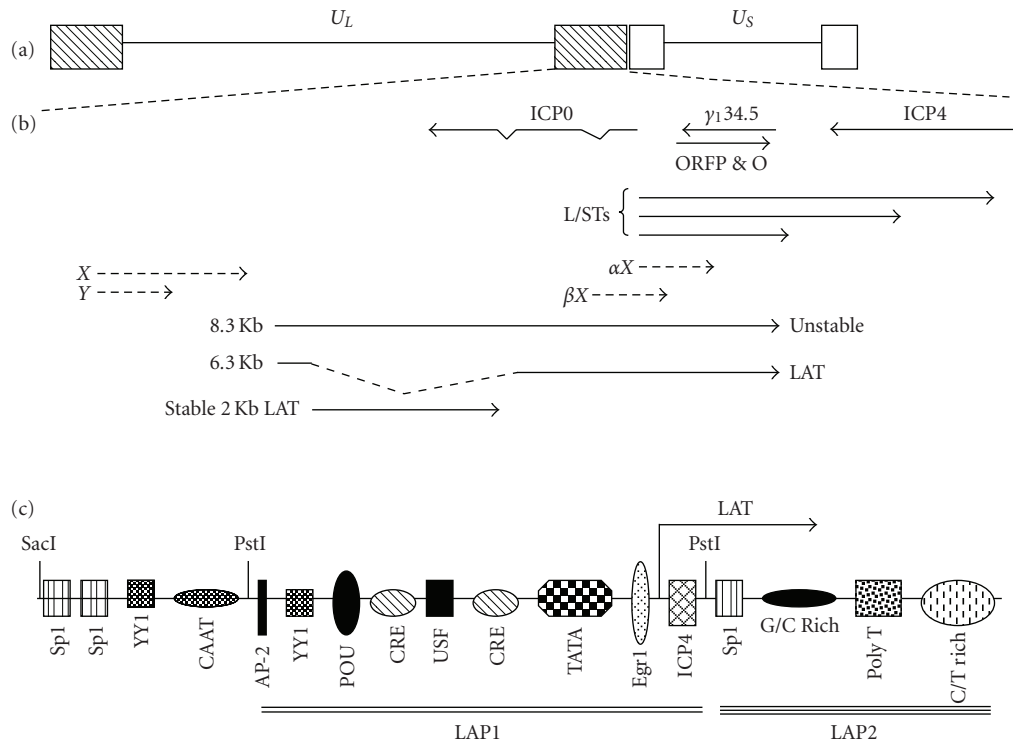


FIGURE 2: Location of genes within the HSV-1 repeats. (a) U_L and U_S denote the unique sequences of the long (L) and short (S) components of the genome. The boxes depict repeat sequences. (b) Transcription map of the repeat region. Location and orientation of LAT [114, 115], ICP0, $\gamma_{134.5}$ [116, 117], ORFP [118], L/STs [119] are indicated by solid lines. Partially mapped transcripts (αX and βX) are denoted by dashed arrows [120, 121]. (c) The LAT promoter contains numerous cis-acting sites that can be bound by cellular transcription factors. Binding of ICP4 to the ICP4 binding site in the LAT promoter inhibits promoter activity [107]. In transient transfection assays, the LAT promoter can be divided into a strong promoter (LAP1) and a weaker promoter (LAP2) [99, 100]. For details of transcripts encoded by LAT, see Figure 3.

are inhibited by another LAT miRNA, miR-H2-3p. The authors conclude that suppression of ICP0 and ICP4 by these miRNAs “facilitates the establishment and maintenance of viral latency.” Since the six LAT-specific miRNAs are not located within the first 1.5 kb of LAT coding sequences, they may only play a supportive role during the latency-reactivation cycle in small animal models of infection. The fact that LAT-specific miRNAs inhibit ICP0 or ICP4 suggests that they enhance the establishment or maintenance of latency. In the context of the latency-reactivation cycle in small animal models, it is unlikely, they are crucial when compared to the first 1.5 kb of LAT coding sequences.

Two additional small RNAs (s-RNAs) are encoded within the first 1.5 kb of LAT coding sequences (LAT s-RNA1 and s-RNA2) [138] (Figure 3(b)). Expression of LAT s-RNA1 and s-RNA2 is readily detected in trigeminal ganglia of latently infected mice [165]. LAT s-RNA2 inhibits ICP4 protein expression, but not RNA expression. LAT s-RNA1 inhibits productive infection approximately 1,000-fold in transient transfections assays, whereas LAT s-RNA2 only inhibits productive infection 5-fold [165]. These LAT s-RNAs may not be miRNAs because they lack Dicer cleavage sites and a mature miRNA band that migrates between 21 and 23 nucleotides was not detected. LAT s-RNA1 and s-RNA2 would not have been identified using the methods described

by Umbach et al. [136] because they size selected RNA species migrating between 17 and 30 nucleotides, and LAT s-RNA1 is 62 nt long and LAT s-RNA2 is 36 nt long.

2.3.3. Novel Transcripts Are Expressed within LAT Coding Sequences. Sequences that encompass LAT also encode several additional transcripts. For example, novel transcripts within the LAT promoter region have been reported [166]. More recently, a transcript and protein, UOL (Upstream of LAT), was identified that is encoded within the LAT promoter regulatory region [167]. Deletion of UOL does not dramatically reduce the spontaneous reactivation phenotype in rabbits [168]. Another transcript, antisense to LAT (AL), is expressed within the first 1.5 kb of LAT coding sequences and the start site of the LAT promoter and appears to encode a protein [169] (see Figure 3 for location of UOL and AL).

Two small ORFs that are also antisense to LAT (AL2 and AL3) have been identified within the first 1.5 kb of LAT coding sequences (Figure 3(b)). A transcript within the first 1.5 kb of LAT coding sequences (AL3) is expressed during productive infection and in trigeminal ganglia of latently infected mice [170]. Like AL, AL3 is antisense with respect to LAT. An AL3 protein was also detected in cells transfected with an AL3 expression vector, and in trigeminal ganglia of infected mice. Conversely, an AL3 protein was not detected

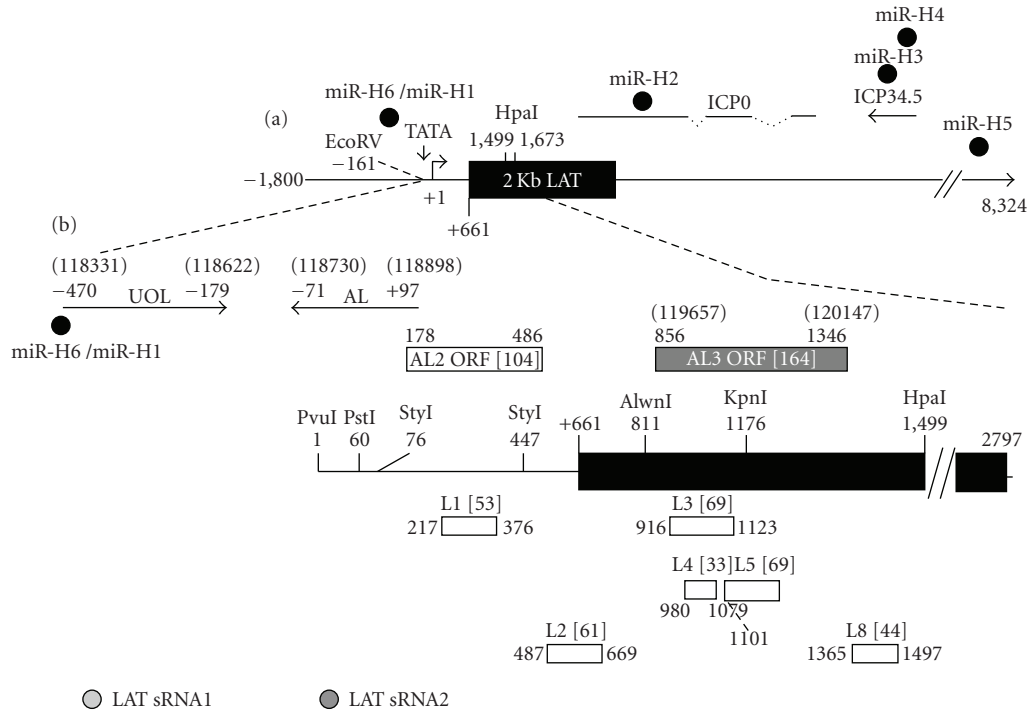


FIGURE 3: Schematic of factors encoded within the LAT locus. (a) Schematic of genes within the long repeats that contain the LAT locus. The large arrow indicates the primary LAT transcript. The solid rectangle represents the very stable 2 kb LAT intron. The start of LAT transcription is indicated by 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 locations of the 6 micro-RNAs (miR-H1-6) that are located within the 8.3 kb LAT [136] are shown. (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 [137]. The numbering system of the ORFs was consistent with a previous study [137]. 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 [138]. Positions of UOL transcript, AL transcript, and ORFs located on the opposite strand of LAT (AL2 and AL3) are shown. The numbers of amino acids of AL2 and AL3 are in brackets. Nucleotide positions relative to the start of LAT transcription are not shown in parenthesis. Numbers in parentheses represent HSV-1 nucleotide positions.

during productive infection, in part, because the 5' terminus of the AL3 transcript is downstream of the first in frame methionine of AL3. It is not currently known whether a transcript encompassing AL2 is expressed during productive infection or during latency. It remains to be seen whether AL2 or AL3 plays a role in the latency-reactivation cycle of HSV-1.

2.4. LAT Regulates the Latency-Reactivation Cycle. As discussed above, the latency-reactivation cycle of HSV-1 can be operationally defined in 3 steps: establishment of latency, maintenance of latency, and reactivation from latency (summarized in Figure 1). In a human being, latency is maintained for the life of the host, indicating that a well-conceived strategy exists that allows for periodic reactivation, while maintaining the viral genome in sensory neurons.

Numerous HSV-1 mutants that do not express detectable levels of LAT have been constructed and tested in animal models [17, 171]. Although a couple of studies have suggested that LAT plays no role in a latent infection [172, 173], most have concluded that LAT is important but not required.

LAT enhances establishment of latency in mice [174, 175] because certain LAT-mutants contain lower levels of viral DNA in murine TG relative to wild type virus [21, 176]. Furthermore, LAT enhances establishment of latency in the rabbit eye model and consequently reduces reactivation from latency [177]. The finding that LAT represses productive viral gene expression in TG of mice during acute infection [178, 179] supports the studies concluding that LAT facilitates establishment of latency. When considering the role that LAT plays in reactivation from latency, its role in establishing latency must be taken into consideration.

LAT enhances establishment of latency in mice [174, 175] or rabbits [180] because certain LAT mutants contain lower levels of viral DNA in TG relative to wt virus [21, 176]. LAT represses productive viral gene expression in TG of mice during acute infection [178, 179] supporting the concept that LAT facilitates establishment of latency. The HSV-1 McKrae strain is frequently shed in tears of infected rabbits as a result of spontaneous reactivation, and LAT is crucial for spontaneous reactivation [177, 181–184]. Furthermore, HSV-1 17syn+ strains with deletions in LAT coding sequences do not reactivate efficiently using the

rabbit eye model [185, 186]. Although LAT overlaps the ICP0 transcript, LAT sequences that promote the latency-reactivation cycle in rabbits do not overlap ICP0 [132].

LAT is also important for *in vivo* reactivation using two different rabbit eye infection models. The McKrae strain of HSV-1 is frequently shed in the tears of infected rabbits as a result of spontaneous reactivation [177, 181, 183, 184, 187]. In contrast, spontaneous reactivation is severely impaired if the LAT gene is deleted. However, these same LAT-mutants grow with the same efficiency as wild-type virus in cultured cells and in ocular tissue of infected rabbits. The first 1.5 Kb of the gene encoding LAT is sufficient for spontaneous reactivation from latency [177] (Figure 3). Since this region does not overlap ICP0, antisense repression of ICP0 expression by LAT does not appear to be required for spontaneous reactivation in the rabbit model. HSV-1 17syn+ strains that have deletions in the LAT promoter and 5' region of the gene encoding LAT (approximately 1,200 base pair) also do not reactivate efficiently in a rabbit eye model [185, 186].

It is not clear whether LAT encodes a protein that regulates the latency-reactivation cycle. Although certain studies suggested that LAT does not encode a protein [137], several studies have concluded that a protein encoded within LAT sequences is expressed [118, 167, 188–192]. These proteins were suggested to substitute for ICP0 functions [191, 192], interfere with binding of ICP4 to DNA [190], or their functions were not described. These proposed LAT proteins map downstream of the critical first 1.5 kb of the primary LAT transcript, a region that appears both sufficient and necessary for LAT's antiapoptosis activity and its ability to support a wild type spontaneous reactivation phenotype [177, 193]. Within the first 1.5 kb of LAT coding sequences, 8 potential ORFs have been identified in the strain McKrae [137] (summarized in Figure 3(b)). A recent study has provided evidence that L2 (Figure 3(b)), which is located in the first 1.5 kb of LAT coding sequences, appears to be expressed in TG of latently infected mice [194]. In summary, the gene encoding LAT does not appear to be absolutely required for latency in small animal models. However, the importance of LAT may be underestimated using small animal models and measuring latency in terms of weeks or months, not decades. The involvement of a LAT encoded protein in the latency-reactivation cycle is unclear.

2.5. HSV-1 Encodes Several Genes That Regulate Apoptosis

2.5.1. Genes Expressed during Productive Infection Inhibit Apoptosis. Many viruses induce apoptosis in cultured cells [195–198]. Killing of infected cells by apoptosis *in vivo* can reduce inflammation, alter immune recognition, reduce burst size, and thus prevent virus spread. Members of the *Alphaherpesvirinae* subfamily induce apoptosis after infection of cultured cells [199–202]. HSV-1 can also induce or inhibit apoptosis in a cell type dependent manner after infection of cultured cells [200, 201, 203–205]. Several antiapoptotic genes encoded by HSV-1 (ICP27, U_s3 , U_s5 , gJ, gD, and LAT) have been identified [200, 201, 203, 204, 206–212]. U_s3 is a protein kinase that, in the absence of other

HSV-1 proteins, inhibits cleavage of BAD and formation of the proapoptotic form of BAD. U_s3 is the only viral protein required for preventing caspase 3 activation, which is the “point of no return” following apoptosis induction. The presence of several HSV-1 antiapoptotic genes suggests that they have specific roles following infection of humans.

HSV infection can induce apoptosis by several distinct mechanisms. For example, HSV induces DNA damage, even in the absence of productive infection [213–217]. DNA damage is a potent stimulus for apoptosis [217]. When expressed from baculovirus expression vectors, $U_s1.5$ and U_L13 can activate caspase 3 [218]. As expected, U_s3 can inhibit the proapoptotic activity of $U_s1.5$ and U_L13 because it can interfere with caspase 3 activation.

2.5.2. LAT Inhibits Apoptosis. LAT interferes with apoptosis in transiently transfected cells and TG of infected mice or rabbits [139, 219–221]. LAT expressing plasmids inhibit caspase 8- and caspase 9-induced apoptosis [222, 223], the two major apoptotic pathways in mammals [224–226]. LAT also inhibits caspase 3 activation [227]. The antiapoptosis functions of LAT correlate with promoting spontaneous reactivation [219, 222]. In fact, inhibiting apoptosis appears to be the most important function of LAT because three different antiapoptosis genes [228–231] restore wt levels of spontaneous reactivation to a LAT null mutant.

LAT s-RNA1 and s-RNA2 (Figure 3) cooperate to inhibit cold-shock-induced apoptosis in transiently transfected mouse neuroblastoma cells [165]. Introduction of ATG → TTG mutations in ORFs within the first 1.5 kb of LAT coding sequences impairs the antiapoptotic functions of LAT [232] suggesting that LAT 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 both small RNAs inhibits their ability to inhibit apoptosis [165]. Although this suggests that the LAT sRNAs mediate the antiapoptotic functions of the first 1.5 kb of LAT coding sequences, there may be additional functions within this region that have antiapoptosis functions.

2.6. Model Describing How LAT Regulates the Latency-Reactivation Cycle. Based on published studies, a working model has been devised to explain how LAT regulates the latency-reactivation cycle. During acute infection of TG (1–4 dpi), extensive viral gene expression occurs [70–72]. The toxic effects of HSV-1 infection, in particular ICP0 [233, 234], $U_s1.5$, and U_L13 [218], make neurons vulnerable to damage and death. The ability of HSV to induce DNA damage [213, 215, 216, 235] would also stimulate the mitochondrial pathway of apoptosis [217]. The antiapoptotic properties of U_s3 , U_s5 , gD, gJ, ICP27, and LAT would promote neuronal survival during acute infection [139, 165, 200, 201, 203, 204, 209, 219–223]. Deletion of LAT might not have a dramatic effect on apoptosis frequency during the early stages of acute infection because the other viral antiapoptotic genes are expressed.

During transition from acute infection to latency (establishment of latency), viral gene expression is extinguished.

The ability of the LAT micro-RNAs to inhibit ICP0 and ICP4 proteins expression [136] as well as the ability of LAT sRNA1 and LAT sRNA2 to inhibit productive infection [165] are likely to promote the establishment of latency. Furthermore, LAT would be the only viral antiapoptotic gene abundantly expressed during the establishment of latency. Neurons in which extensive viral gene expression had occurred during acute infection (permissive neurons) would be vulnerable to apoptosis in the absence of LAT expression. Nonpermissive neurons that harbor viral genomes would have suffered low levels of viral induced damage and thus would have a higher probability of survival in the absence of LAT. In mice, subsets of neurons have been identified in TG and the ability of HSV-1 to infect these neurons is different [236], supporting the concept that permissive and nonpermissive neurons exist.

The antiapoptosis functions of LAT would also appear to be crucial for protecting neurons from apoptotic stimuli during the maintenance of latency because it is the only viral gene that is abundantly expressed (Figure 1). In fact, during latency, LAT does have an effect on the number of surviving neurons following infection of mice [237]. Furthermore, the ability of LAT micro-RNAs [136] and LAT sRNA1 or LAT sRNA2 [165] to inhibit viral gene expression and/or productive infection would promote maintenance of latency. Since LAT sRNA1 and LAT sRNA2 [165] are located within the first 1.5 kb of LAT coding sequences, these small RNAs appear to be more important than the LAT micro-RNAs. However, the ability of the respective LAT noncoding RNAs to inhibit viral gene expression or productive infection is not as important as inhibiting apoptosis because three different antiapoptosis genes restore wt levels of spontaneous reactivation to a LAT null mutant [228–231].

The response of the central or peripheral nervous system to trauma, stress, or immunosuppression plays an important role during reactivation from latency. Stress leads to elevated corticosteroid levels, which has rapid effects on neural activity [238, 239]. Dexamethasone, a synthetic corticosteroid, induces viral gene expression [240], stimulates an HSV-1 origin of replication (Ori-L) in neuronal cells [50], and alters splicing patterns in the absence of protein synthesis [241]. Corticosteroids, or other forms of stress or trauma can induce neuronal neurodegeneration and/or apoptosis [242–248]. Since reactivation induces productive gene expression, all HSV-1 antiapoptotic genes would be expressed and should prolong neuronal survival, thus enhancing virus production.

2.7. Cell-Mediated Immune Responses Are Important for the Latency-Reactivation Cycle

2.7.1. Infiltration of Lymphocytes to TG during Acute Infection.

Several independent studies have demonstrated that T cells, CD8⁺ T lymphocytes in particular, are crucial for controlling HSV infection in sensory ganglia [249, 250]. During acute infection, HSV antigen expression increases until 3 dpi in TG but is undetectable at 7 dpi [251]. Coincident with a decline of HSV antigen in TG there is an increase in Mac-1⁺ cells, macrophages, natural killer cells (NK), and certain CD8⁺

cells. No cells with characteristic lymphoid cell morphology can be detected in uninfected TG. After 5 dpi, the number of CD8⁺ T cells, F4/80⁺ cells (macrophages), and $\gamma\delta$ T cells increases dramatically. At 3 dpi, TG neurons that are viral antigen positive can be detected that are surrounded by nonneural cells expressing TNF- α , IL-6, or IFN- γ [252]. Cells that express IL-2 or IL-4 are detected later after infection when viral antigens are difficult to detect. The number of cells producing IFN- γ and IL-4 increases between 3 and 7 dpi but the same cells do not appear to produce both factors [251]. At 7 days after infection, transcripts encoding IL-2, IL-10, IFN- γ , TNF- α , or RANTES (regulated upon activation, normal T cell expressed and secreted mRNA) are detected by RT-PCR [253]. By ELISA, IL-2, IL-6, IL-10, and IFN- γ are detected at the same time confirming the RT-PCR results. The same cellular antigens were not detected in TG from uninfected mice indicating that these changes were induced by infection.

2.7.2. Persistence of Lymphocytes in the Peripheral Nervous System during Latency.

If true latency of HSV is established, cytokine expression in TG would not be detected. However, several studies have concluded that a persistent cell-mediated immune response occurs in TG during latency, and that T cells, CD8⁺ T lymphocytes in particular, inhibit reactivation from latency [249–251, 254–258].

The obvious explanation for persistence of immune effector cells in TG is that low levels of viral proteins are expressed and an immune response occurs. A careful examination of TG neurons for viral gene expression in HSV-1 latently infected mice (37–47 days after infection) demonstrated that abundant viral transcripts, viral protein, and viral DNA replication occur in approximately 1 neuron per 10 TG [259]. Infectious virus is not detected in these mice confirming that they were latently infected. Neurons expressing high levels of HSV-1 transcripts are invariably surrounded by foci of infiltrating white blood cells. The term “spontaneous molecular reactivation” has been coined to describe these rare neurons [259].

2.7.3. Interferon Can Inhibit Reactivation from Latency.

Persistence of the immune system in TG during latency is believed to play a role in the latency-reactivation cycle. CD8⁺ T cells that produce interferon- γ play an important role in preventing reactivation from latency in sensory neurons in mice latently infected with HSV-1 [256, 257]. Two independent studies have also concluded that interferon- α and interferon- γ control recurrent herpetic lesions [260, 261]. In addition to interferon, lymphocyte-mediated cytotoxicity could inhibit virus spread in TG. Lymphocyte-mediated cytotoxicity induces two potent apoptotic pathways: the granule exocytosis and the Fas-Fas ligand pathways [262, 263]. The granule exocytosis pathway is employed predominantly by CD8⁺, natural killer, and lymphokine-activated killer cells. A recent study has demonstrated that release of granzyme B from CD8⁺ T cells into latently infected neurons helps to inhibit reactivation from latency by cleaving ICP4 [264]. Since it is well established that granzyme B activates

caspase 3 and the intrinsic pathway of apoptosis [265], the ability of LAT to inhibit apoptosis during maintenance of latency appears to be important.

3. Conclusions

HSV-1 latency is a complicated virus host interaction that is crucial for virus transmission, survival in nature, and recurrent disease. Numerous studies have indicated that sensory neurons are the primary site for latency. Since LAT is abundantly expressed in latently infected neurons, it is not surprising to find that LAT is important for the latency-reactivation cycle in small animal models. It is currently not clear whether expression of a LAT protein is important. Given the fact that several LAT small RNAs, including 6 known micro-RNAs, are expressed during latency implies that these small nonprotein coding RNAs are important for life-long latency in humans. The finding that LAT sRNA1 and sRNA2 cooperate to inhibit apoptosis and also can inhibit productive infection supports a regulatory role for these small RNAs during the latency-reactivation cycle. It is also possible that additional transcripts encoded within LAT coding sequences (AL, AL3, or UOL) play a role in the latency-reactivation cycle. It will be necessary to design viral mutants that do not express these respective factors and then test the ability of these viruses to reactivate from latency in small animal models of infection.

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