Journal of NeuroVirology, 13: 78–84, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280601164333

informa
healthcare

Short Communication

Reactivation phenotype in rabbits of a herpes simplex virus type 1 mutant containing an unrelated antiapoptosis gene in place of latency-associated transcript

Ling Jin,[1] Guey-Chuen Perng,[1] Dale Carpenter,[1] Kevin R Mott,[1] Nelson Osorio,[1] Julia Naito,[1] David J Brick,[1] Clinton Jones,[2] and Steven L Wechsler[1]

[1] *The Eye Institute, University of California Irvine, School of Medicine, Irvine, California, USA;*
[2] *Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, Nebraska, USA*

Latency-associated transcript (LAT) significantly enhances the spontaneous reactivation phenotype of herpes simplex virus type 1 (HSV-1). The mechanism by which LAT accomplishes this has been elusive. To determine if LAT's antiapoptosis activity is involved, the authors used a rabbit eye model to analyze the spontaneous reactivation phenotype of an HSV-1 mutant in which LAT was replaced by an unrelated antiapoptosis gene. This virus, dLAT-cpIAP, contains the open reading frame of the baculovirus inhibitor of apoptosis protein gene (cpIAP) in place of LAT, under control of the LAT promoter. The authors report here that in a rabbit ocular model of infection, dLAT-cpIAP had a spontaneous reactivation phenotype similar to wild-type virus and significantly higher than LAT(−) viruses. This was consistent with their previous findings using the mouse trigeminal ganglia explant–induced reactivation model. Whether LAT (and in the case of dLAT-cpIAP, cpIAP) enhances the spontaneous reactivation phenotype by functioning during establishment of latency, maintenance of latency, or reactivation from latency, or during two or more of these periods, remains to be determined. Regardless, the results presented in this study strongly support the hypothesis that LAT's antiapoptosis activity is the dominant function that enhances HSV-1's spontaneous reactivation phenotype. *Journal of NeuroVirology* (2007) **13,** 78–84.

Keywords: apoptosis; HSV-1; latency; latency-associated transcript

Herpes simplex virus type 1 (HSV-1) is a neurotropic virus. Primary acute infection usually occurs at a mucosal membrane and is usually asymptomatic or produces mild clinical symptoms. During the acute infection the virus infects sensory nerve endings, travels up the sensory neurons to the nerve bodies, and ultimately establishes a lifelong latent infection in the nucleus of ganglionic sensory neurons. Sporadic reactivations can occur throughout life. The virus can then travel back down axons to the original peripheral site where infectious virus can be shed. Shedding of reactivated virus can be completely asymptomatic or can result in moderate to very severe recurrent herpetic disease (Fleming *et al*, 1997; Wald *et al*, 2000). Recurrent HSV-1 can cause painful genital lesions, cold sores in and around the mouth, neurological complications including encephalitis, and potentially blinding disease of the cornea, known as herpetic stromal keratitis (HSK). Sixty percent to 90% of adults in the developed world harbor latent HSV-1 (Whitley, 1996). Recent data

Address corresponence to Dr. Steven L. Wechsler, The Eye Institute, Department of Ophthalmology, University of California Irvine, School of Medicine, Irvine, CA 92697, USA. E-mail: Wechsler@uci.edu

This work was supported by Public Health Service grants EY13191, EY12823, and P20RR15635; USDA grants 2005-01554 and 2006-01627; The Discovery Eye Foundation; The Henry L. Guenther Foundation; and Research to Prevent Blindness. Dr. Wechsler is an RPB Senior Scientific Investigator.

Received 17 October 2006; revised 8 November 2006; accepted 21 November 2006.

indicate that of adults in the United States between the ages of 30 and 49, over 24% are seropositive for HSV-2 and over 64% are seropositive for HSV-1 (Xu *et al*, 2006). Over 450,000 people in the United States have a history of recurrent corneal disease (Liesegang, 1989; Liesegang *et al*, 1989; Whitley and Roizman, 2001).

Latently infected individuals can shed reactivated virus at a rate of 10% (i.e., infectious virus can be detected at the peripheral location 1 day out of 10), even in the complete absence of clinical symptoms. HSV is an ancient human virus, coevolving with and in the human population for hundreds of thousands of years. The ability of the virus to establish latency and shed virus at high rates for the life of the individual, usually in the absence of clinical disease, appears to be an evolutionary adaptation that allows the virus to infect the maximum number of individuals without killing or incapacitating its host population. Because both virus spread within the human population and most of the severe disease due to herpes simplex virus are associated with reactivation of the virus from latency, understanding the molecular events and mechanisms of the HSV-1 latency-reactivation cycle is an area of intense interest and study.

Of the 80 plus HSV-1 genes, the latency-associated transcript (LAT) locus is the only genomic region from which a high level of transcription is consistently detected during neuronal latency (Deatly *et al*, 1987; Rock *et al*, 1987; Stevens *et al*, 1987). LAT mutants that do not express detectable amounts of LAT have a reduced or delayed reactivation phenotype in mice and a reduced reactivation phenotype in rabbits (Block *et al*, 1993; Devi-Rao *et al*, 1994; Hill *et al*, 1990; Leib *et al*, 1989; Perng *et al*, 1994, 2001b; Sawtell and Thompson, 1992; Steiner *et al*, 1989). Thus, LAT plays an important role in the HSV-1 latency-reactivation cycle. It remains unclear whether LAT's major impact is during the reactivation phase, during maintenance of latency, during establishment of latency, or a combination of two or more of these periods. The mechanism by which LAT enhances the reactivation phenotype is also not fully elucidated and is controversial. However, it is unequivocal that LAT plays an important role in latency.

It is now generally accepted that LAT has antiapoptosis activity and this is a likely candidate for a functional mechanism by which LAT might enhance the latency-reactivation cycle (Ahmed *et al*, 2002; Gupta *et al*, 2006; Inman *et al*, 2001b; Perng *et al*, 2000a, 2002). We have shown that the LAT reactivation phenotype in rabbits and mice and LAT's antiapoptosis activity map to within the same region of the LAT transcript, namely the first 1.5 kb of the primary 8.3- to 8.5-kb LAT RNA, approximately the first 20% of the primary transcript (Inman *et al*, 2001b; Jin *et al*, 2003). This strongly suggests that LAT's antiapoptosis activity and LAT's ability to enhance the reactivation phenotype are related and specifically supports our hypothesis that LAT's antiapoptosis activity plays a major role in the mechanism by which LAT enhances the reactivation phenotype.

To further test the above hypothesis, we previously constructed the chimeric virus, CJLAT, in which the bovine herpes virus Latency Related (LR) gene replaced both copies of the first 1.5 kb of the HSV-1 LAT gene in an otherwise HSV-1 McKrae genomic background (Perng *et al*, 2002). Although LR is the bovine herpes analog of LAT, there is no sequence homology between these genes. Although LR and LAT have both been shown to block apoptosis, LR does so via an LR protein (Ciacci-Zanella *et al*, 1999), whereas no antiapoptosis LAT protein has yet been identified (Drolet *et al*, 1998). CJLAT has a wild-type (i.e., LAT(+)) reactivation phenotype in both mice and rabbits that is dependent on expression of the LR protein (Mott *et al*, 2003). However, because LR is the analog of LAT, it remained possible that LR and LAT share an as yet undetermined function in addition to antiapoptosis activity and that it was this other function that was critical for LAT's ability to enhance the reactivation phenotype.

We therefore constructed another chimeric virus, dLAT-cpIAP, in which the functional region of LAT was replaced with the well-characterized baculovirus antiapoptosis gene cpIAP. We found that dLAT-cpIAP has a wild-type LAT(+)-like induced reactivation phenotype in the mouse TG explant model (Jin *et al*, 2005). Here we report that dLAT-cpIAP also has a wild-type LAT(+)-like spontaneous reactivation phenotype in the rabbit ocular model of HSV-1 latency and reactivation.

The genomic structure of the LAT regions of dLAT-cpIAP and other viruses used or discussed in this report (Jin *et al*, 2005; Perng *et al*, 1994, 2001a, 2000b) are shown schematically in Figure 1. All mutants were derived from wild-type HSV-1 strain McKrae. Although there are two copies of the LAT locus in the HSV-1 genome, one in each viral long repeat, only one copy is shown for simplicity. The numbers indicate nucleotide (nt) positions relative to the start of the primary LAT transcript at +1 (genomic nt 118,801 of the internal long repeat). dLAT-cpIAP (Jin *et al*, 2005) contains the baculovirus cpIAP gene open reading frame (ORF) in place of LAT nt 76 to 1667, driven by the LAT promoter, followed by the simian virus 40 (SV40) polyadenylation signal sequence. cpIAP is a well-characterized inhibitor of apoptosis gene from Baculoviridae *Cydia pomonella* granulovirus (Birnbaum *et al*, 1994; Clem and Miller, 1994; Crook *et al*, 1993). In large part, cpIAP blocks apoptosis by binding to caspase 9 (Huang *et al*, 2000; Vilaplana and O'Reilly, 2003).The region of LAT after the polyA site is represented by dashed lines to indicate that no downstream LAT transcription is detected. dLAT2903 contains a deletion in both copies of LAT from nt −161 to +1667. This virus is missing key promoter elements, makes no detectable LAT RNA (indicated by dashed line), is a true LAT-null mutant, and has a low reactivation phenotype

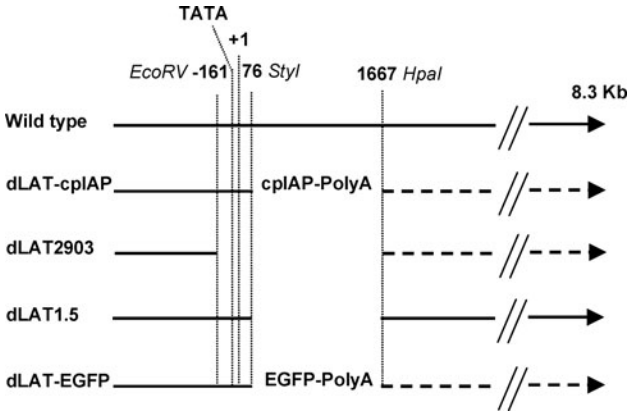

Figure 1 Genomic structure of the LAT locus in HSV-1 mutants. Wild type: The primary LAT transcript is approximately 8.3 kb. LAT transcription starts at +1 corresponding to genomic nt 118,801. dLAT-cpIAP contains the antiapoptosis gene cpIAP followed by a poly-A signal inserted in place of LAT nt 76 to 1667. The entire LAT promoter is present. No LAT RNA is transcribed past the poly-A site. dLAT2903 is deleted from LAT nt −161 to +1667 and is a true LAT-null mutant that is missing primary LAT promoter elements between −161 and +1 and a putative secondary LAT promoter, LAP2, located within the 5′ end of the primary LAT transcript (Goins *et al*, 1994). This mutant therefore is not capable of expressing any LAT RNA (dashed lines) (Perng *et al*, 1994). dLAT1.5 is identical to dLAT-cpIAP, but with no inserted ORF or polyadenylation signal. The region of LAT past the deletion is transcribed normally. dLAT-EGFP is identical to dLAT-cpIAP except that the irrelevant gene EGFP is inserted instead of cpIAP.

compared to wild-type HSV-1 (Perng *et al*, 1994). dLAT-EGFP (Perng *et al*, 2000b) (EGFP = enhanced green fluorescent protein) is identical to dLAT-cpIAP except that it contains the EGFP ORF in place of the cpIAP ORF. dLAT1.5 (Perng *et al*, 2001a) is identical to dLAT-cpIAP and dLAT-EGFP except that it does not contain any inserted ORF nor a polyadenylation signal sequence. dLAT1.5 transcribes LAT RNA downstream of the deletion.

We previously showed that dLAT-cpIAP, containing the unrelated antiapoptosis gene cpIAP in place of LAT, (1) is indistinguishable from wild-type virus in terms of replication in tissue culture, replication in mouse eyes, and replication in mouse trigeminal ganglia (TG); (2) expresses the cpIAP RNA with wild type LAT-like kinetics; (3) expresses a functional cpIAP protein (i.e., the cpIAP protein retains its antiapoptosis activity); and (4) has a LAT(+)-like (i.e., wild type-like) explant TG–induced reactivation phenotype in the mouse ocular model that is significantly higher than that of LAT(−) viruses (Jin *et al*, 2005). These results indicated that the unrelated antiapoptosis gene cpIAP can efficiently substitute for the LAT function involved in enhancing the mouse explant TG–induced reactivation phenotype. To determine if cpIAP can also efficiently substitute for the LAT function involved in enhancing the spontaneous reactivation phenotype and to extend these results to a second animal model, we examined the spontaneous reactivation phenotype of dLAT-cpIAP in a rabbit ocular model of HSV-1 spontaneous reactivation. The rabbit ocular model of HSV-1 latency and reactivation may be a better reflection of the clinical situation because spontaneous reactivation of HSV-1 occurs in rabbits at a rate similar to that in humans, whereas spontaneous reactivation does not occur in mice. In addition, the HSV-1–induced corneal disease in rabbits closely resembles that seen in humans, whereas the corneal disease in mice is dissimilar. It is also important to study the reactivation phenotype in more than one small animal model as not all LAT mutants affect the reactivation phenotype similarly in mice and rabbits (Hill *et al*, 1996; Maggioncalda *et al*, 1996).

Twenty rabbits per group were infected with 2×10^5 plaque-forming units (pfu)/eye of dLAT-cpIAP, dLAT2903, or wild type McKrae. The viruses were delivered as eye drops in 25 μl of tissue culture medium, without corneal scarification (i.e., without first scratching the cornea) as we previously described (Perng *et al*, 1994). To first examine replication of dLAT-cpIAP in rabbit eyes, tears were collected from one eye of 10 different rabbits at the times indicated in Figure 2A and B. dLAT2903 was not included because replication of dLAT2903 and wild-type McKrae are indistinguishable in rabbit eyes (Perng *et al*, 1994). Although dLAT-cpIAP was previously shown to replicate similarly to wild type in tissue culture and mouse eyes (Jin *et al*, 2005), it appeared to replicate less efficiently than wild type in rabbit eyes (Figure 2A and B). Although this was unexpected, there are other examples of LAT-related mutants that have significantly different phenotypes in rabbits compared to mice (Loutsch *et al*, 1999; Perng *et al*, 2001a). The specific reasons remain unknown but are likely due to differences in virus-host interactions in the respective small animal models. This might result in decreased virus load in trigeminal ganglia and fewer neurons becoming latently infected compared to wild type–infected rabbits. Unfortunately, for technical reasons, it is very difficult to accurately quantitate the amount of viral DNA or the amount of latency in rabbit trigeminal ganglia. In addition, if the spontaneous reactivation phenotype of dLAT-cpIAP is similar to wt then this is not critical.

Relative spontaneous reactivation rates were determined by analysis of serum neutralizing antibody titers. Serum neutralizing antibody titers are a sensitive measure of detecting dexamethasone induced reactivation of calves latently infected with bovine herpes virus (BHV-1) (Inman *et al*, 2001a; Jones *et al*, 2000), indicating that an increase in virus specific antibodies normally occurs following α-herpesvirus reactivation. Similarly, although neutralizing antibody titers are similar during the first 45 days post infection (p.i.) in rabbits infected with viruses having either efficient (LAT(+), wild type) or inefficient (LAT(−)) spontaneous reactivation phenotypes, in rabbits latently infected with an efficiently reactivating virus, the neutralizing antibody titers continue to increase

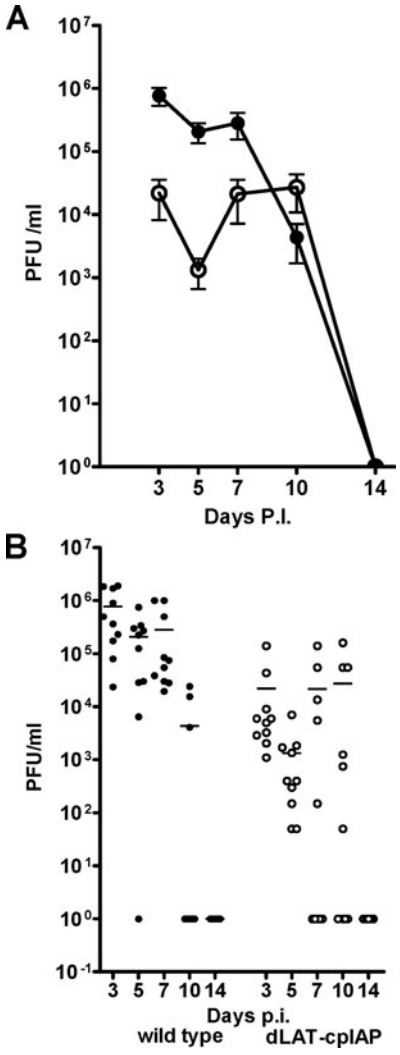

Figure 2 Replication of dLAT-cpIAP in rabbit eyes. **A**, Tears were collected at the times indicated and the amount of infectious virus was determined by standard plaque assays on rabbit skin (RS) cells as previously described (Perng *et al*, 1994). The means ± SD are shown. Open circles = dLAT-cpIAP; solid circles = wild type. **B**, The virus titer for each eye at each time is shown to give a visual representation of the scatter common in this system.

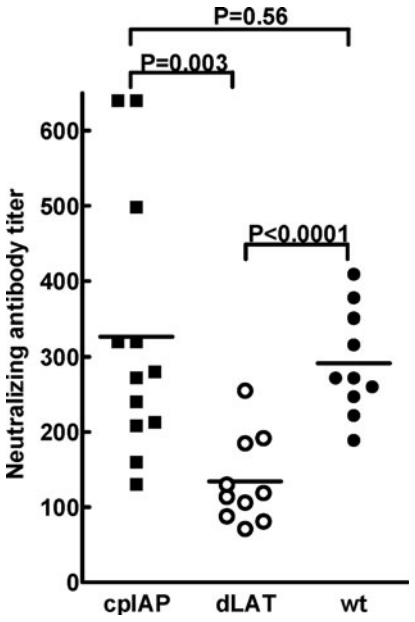

Figure 3 Relative spontaneous reactivation of dLAT-cpIAP estimated by HSV-1 serum neutralizing antibody titers. Rabbits were infected with 2×10^5 pfu/eye of dLAT-cpIAP (cpIAP), dLAT2903 (dLAT), or wild-type McKrae (wt). Serum was collected on day 59 pi and neutralizing antibody titers determined on individual sera by standard 50% plaque reduction assays as previously described (Perng *et al*, 1999). The horizontal bars indicate the mean titer for each group.

after day 45 p.i., whereas in rabbits latently infected with poorly reactivating viruses, the neutralizing antibody titers fall after day 45 p.i (Perng *et al*, 1999). By day 59 p.i. (approximately 31 to 45 days after latency is established), this difference is readily apparent and it can be used to determine the relative spontaneous reactivation phenotype (Perng *et al*, 2002; Perng *et al*, 2000b).

Using this approach, sera were collected on day 59 p.i. from 12 dLAT-cpIAP, 10 dLAT2903, and 10 wild-type latently infected rabbits, and neutralizing antibody titers were determined for individual sera by 50% plaque reduction assays as previously described (Perng *et al*, 2000b) (Figure 3). The average neutralizing antibody titer of sera from the dLAT-cpIAP latently infected rabbits was significantly higher than that of dLAT2903 ($P = .003$; t test) and similar to that of wild type ($P = .56$). This indicated that dLAT-cpIAP had a high LAT(+)-like spontaneous reactivation phenotype.

As summarized in Table 1, we previously showed that in the rabbit model dLAT1.5 has a LAT(−)-like reactivation phenotype (Perng *et al*, 2001a). dLAT1.5 contains the same LAT deletion as dLAT-cpIAP (LAT nt 76 to 1667) but does not contain any foreign DNA insert (Perng *et al*, 2001a) (see Figure 1). In addition, dLAT1.5 transcribes all of LAT past LAT nt 1667 (i.e., past the location of the cpIAP-polyadenylation

Table 1 Relative spontaneous reactivation of dLAT-cpIAP, dLAT-EGFP, and dLAT1.5

Virus	*Percent of wild-type McKrae spontaneous reactivation*	P *versus wild type*
dLAT-cpIAP	112%[a]	.56[d]
dLAT2903	46%[a]	<.001[d]
dLAT-EGFP	35%[b]	.002[d]
dLAT1.5	52%[c]	.003[e]

[a]Based on neutralizing antibody titers on day 59 post infection from data in Figure 3.
[b]Based on neutralizing antibody titers on day 60 (Perng *et al*, 2000b).
[c]Based on the fraction of virus-positive tear film cultures during latency (Perng *et al*, 2001a).
[d]By the Student t test.
[e]By the chi-square test.

site insert in dLAT-cpIAP). Thus, it is unlikely that the LAT(+)-like spontaneous reactivation phenotype of dLAT-cpIAP in rabbits was due to read through transcription past the cpIAP insertion. We also previously showed that dLAT-EGFP has a LAT(−)-like spontaneous reactivation phenotype in rabbits (Perng *et al*, 2000b). dLAT-EGFP is identical to dLAT-cpIAP, except that it contains the EGFP (enhanced green fluorescence protein) gene in place of cpIAP (see Figure 1). Thus, it is unlikely that the LAT(+)-like reactivation phenotype of dLAT-cpIAP was due to insertion of a random DNA sequence at this LAT locus. Thus, neither read through LAT transcription past the cpIAP insertion nor insertion of irrelevant DNA at the site of the cpIAP insertion can account for the high LAT(+)-like reactivation phenotype of dLAT-cpIAP in the rabbit. These results therefore demonstrate that an unrelated antiapoptosis gene (cpIAP) can efficiently substitute for the LAT function involved in enhancing the spontaneous reactivation phenotype of HSV-1 in the rabbit ocular model. As discussed above, we previously showed that cpIAP can also efficiently substitute for the LAT function involved in enhancing the explant TG–induced reactivation phenotype in mice. Within the limits of the mouse and rabbit small animal models, LAT's antiapoptosis activity appears able to completely account for LAT's ability to enhance induced and spontaneous reactivation of HSV-1.

In addition to having a wild type–like reactivation phenotype in mice, the above results demonstrate that dLAT-cpIAP also has a LAT(+)-like wild-type spontaneous reactivation phenotype in the rabbit ocular model. This is important because (1) the explant TG–induced reactivation phenotype in the mouse is not always predictive of the *in vivo* spontaneous reactivation phenotype in rabbits; (2) the spontaneous reactivation rate in the rabbit model is similar to that in humans, whereas spontaneous reactivation in mice occurs not at all, or too rarely to study (Gebhardt and Halford, 2005), suggesting that the rabbit may be a better predictor of the spontaneous reactivation phenotype in humans; and (3) our initial finding that LAT has antiapoptosis activity (Perng *et al*, 2000a) was controversial. Thus, we felt it was important to confirm that dLAT-cpIAP, containing the unrelated antiapoptosis gene cpIAP in place of LAT, could support LAT(+)-like HSV-1 reactivation in the rabbit as well as the mouse.

Two microRNAs (miRNAs) have recently been reported in the LAT region. One is located upstream of LAT transcription (Cui *et al*, 2006) and is therefore unlikely to account for a LAT RNA function. The other has antiapoptosis activity (Gupta *et al*, 2006) and is located within the region of LAT previously shown to enhance the reactivation phenotype and block apoptosis (Ahmed *et al*, 2002; Inman *et al*, 2001b; Jin *et al*, 2003; Perng *et al*, 1996a). If as the authors propose this miRNA accounts for some or all of LAT's antiapoptosis activity, it would be consistent with our previous suggestion that LAT does not encode a protein involved in enhancing the reactivation phenotype (or blocking apoptosis) (Drolet *et al*, 1998). Furthermore, because dLAT-cpIAP and CJLAT (Mott *et al*, 2003) successfully substitute for LAT by expressing an antiapoptosis protein, the mechanism by which apoptosis is inhibited (i.e., via a protein versus via an RNA) may not be significant with respect to the HSV-1 reactivation phenotype in small animal models. However, it is unlikely that the miRNA reported by Gupta *et al* (2006) fully accounts for LAT's function because at least two mutants capable of expressing this miRNA have significantly reduced reactivation phenotypes (Drolet *et al*, 1999), whereas at least one mutant that should be unable to produce this miRNA has a wild-type reactivation phenotype (Perng *et al*, 1996b).

The study presented here and the studies discussed above strongly argue that LAT's antiapoptosis activity is directly involved in the mechanism by which LAT enhances the reactivation phenotype. This does not rule out the possibility that LAT may have additional functions that contribute to the reactivation phenotype, or that might independently be able to support the LAT high reactivation phenotype. Although HSV-1 has other antiapoptosis genes, LAT is the only one that is expressed (1) at the end of the acute infection when latency is being established; (2) throughout latency when latency is being maintained; and (3) when reactivation from latency is triggered. Thus, if blocking apoptosis is critical at any of these times during the latency-reactivation cycle, LAT is the most likely viral gene to provide this function. Currently, whether LAT exerts its major influence during establishment of latency, during maintenance of latency, during reactivation from latency, or during a combination of two or more of these periods remains to be determined.

References

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

Birnbaum MJ, Clem RJ, Miller LK (1994). An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. *J Virol* **68:** 2521–2528.

Block TM, Deshmane S, Masonis J, Maggioncalda J, Valyi-Nagi T, Fraser NW (1993). An HSV LAT null mutant reactivates slowly from latent infection and makes small plaques on CV-1 monolayers. *Virology* **192:** 618–630.

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

Clem RJ, Miller LK (1994). Control of programmed cell death by the baculovirus genes p35 and iap. *Mol Cell Biol* **14:** 5212–5222.

Crook NE, Clem RJ, Miller LK (1993). An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J Virol* **67:** 2168–2174.

Cui C, Griffiths A, Li G, Silva LM, Kramer MF, Gaasterland T, Wang XJ, Coen DM (2006). Prediction and identification of herpes simplex virus 1-encoded microRNAs. *J Virol* **80:** 5499–5508.

Deatly AM, Spivack JG, Lavi E, Fraser NW (1987). RNA from an immediate early region of the type 1 herpes simplex virus genome is present in the trigeminal ganglia of latently infected mice. *Proc Natl Acad Sci U S A* **84:** 3204–3208.

Devi-Rao GB, Bloom DC, Stevens JG, Wagner EK (1994). Herpes simplex virus type 1 DNA replication and gene expression during explant-induced reactivation of latently infected murine sensory ganglia. *J Virol* **68:** 1271–1282.

Drolet BS, Perng GC, Cohen J, Slanina SM, Yukht A, Nesburn AB, Wechsler SL (1998). The region of the herpes simplex virus type 1 LAT gene involved in spontaneous reactivation does not encode a functional protein. *Virology* **242:** 221–232.

Drolet B, Perng G, Villosis R, Slanina S, Nesburn A, Wechsler S (1999). Expression of the first 811 nucleotides of the HSV-1 latency associated transcript (LAT) paritally restores wild type spontaneous reactivation to a LAT null mutant. *Virology* **253:** 96–106.

Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, St Louis ME (1997). Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* **337:** 1105–1111.

Gebhardt BM, Halford WP (2005). Evidence that spontaneous reactivation of herpes virus does not occur in mice. *Virol J* **2:** 67.

Goins WF, Sternberg LR, Croen KD, Krause PR, Hendricks RL, Fink DJ, Straus SE, Levine M, Glorioso JC (1994). A novel latency-active promoter is contained within the herpes simplex virus type 1 UL flanking repeats. *J Virol* **68:** 2239–2252.

Gupta A, Gartner JJ, Sethupathy P, Hatzigeorgiou AG, Fraser NW (2006). Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. *Nature* **442:** 82–85.

Hill JM, Maggioncalda JB, Garza HH Jr, Su YH, Fraser NW, Block TM (1996). In vivo epinephrine reactivation of ocular herpes simplex virus type 1 in the rabbit is correlated to a 370-base-pair region located between the promoter and the 5' end of the 2.0 kilobase latency-associated transcript. *J Virol* **70:** 7270–7274.

Hill JM, Sedarati F, Javier RT, Wagner EK, Stevens JG (1990). Herpes simplex virus latent phase transcription facilitates in vivo reactivation. *Virology* **174:** 117–125.

Huang Q, Deveraux QL, Maeda S, Salvesen GS, Stennicke HR, Hammock BD, Reed JC (2000). Evolutionary conservation of apoptosis mechanisms: lepidopteran and baculoviral inhibitor of apoptosis proteins are inhibitors of mammalian caspase-9. *Proc Natl Acad Sci U S A* **97:** 1427–1432.

Inman M, Lovato L, Doster A, Jones C (2001a). A mutation in the latency-related gene of bovine herpesvirus 1 leads to impaired ocular shedding in acutely infected calves. *J Virol* **75:** 8507–8515.

Inman M, Perng G, Henderson G, Ghiasi H, Nesburn A, Wechsler S, Jones C (2001b). Region of Herpes Simplex Virus type 1 latency-associated transcript sufficient for wild type spontaneous reactivation promotes cell survival in tissue culture. *J Virol* **75:** 3636–3646.

Jin L, Peng W, Perng GC, Brick DJ, Nesburn AB, Jones C, Wechsler SL (2003). Identification of herpes simplex virus type 1 latency-associated transcript sequences that both inhibit apoptosis and enhance the spontaneous reactivation phenotype. *J Virol* **77:** 6556–6561.

Jin L, Perng GC, Mott KR, Osorio N, Naito J, Brick DJ, Carpenter D, Jones C, Wechsler SL (2005). A herpes simplex virus type 1 mutant expressing a baculovirus inhibitor of apoptosis gene in place of latency-associated transcript has a wild-type reactivation phenotype in the mouse. *J Virol* **79:** 12286–12295.

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

Leib DA, Bogard CL, Kosz-Vnenchak M, Hicks KA, Coen DM, Knipe DM, Schaffer PA (1989). A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. *J Virol* **63:** 2893–2900.

Liesegang TJ (1989). Epidemiology of ocular herpes simplex. Natural history in Rochester, Minn, 1950 through 1982. *Arch Ophthalmol* **107:** 1160–1165.

Liesegang TJ, Melton LJ 3rd, Daly PJ, Ilstrup DM (1989). Epidemiology of ocular herpes simplex. Incidence in Rochester, Minn, 1950 through 1982. *Arch Ophthalmol* **107:** 1155–1159.

Loutsch JM, Perng GC, Hill JM, Zheng X, Marquart ME, Block TM, Ghiasi H, Nesburn AB, Wechsler SL (1999). Identical 371-base-pair deletion mutations in the LAT genes of herpes simplex virus type 1 McKrae and 17syn+ result in different in vivo reactivation phenotypes. *J Virol* **73:** 767–771.

Maggioncalda J, Mehta A, Bagasra O, Fraser NW, Block TM (1996). A herpes simplex virus type 1 mutant with a deletion immediately upstream of the LAT locus establishes latency and reactivates from latently infected mice with normal kinetics. *J NeuroVirol* **2:** 268–278.

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

Perng G, Jones C, Ciacci-Zanella H, Henderson G, Yukht A, Slanina S, Hofman F, Ghiasi H, Nesburn A, Wechsler S (2000a). Virus induced neuronal apoptosis blocked by the herpes simplex virus latency associated transcript (LAT). *Science* **287:** 1500–1503.

Perng GC, Dunkel EC, Geary PA, Slanina SM, Ghiasi H, Kaiwar R, Nesburn AB, Wechsler SL (1994). The latency-associated transcript gene of herpes simplex virus type 1 (HSV-1) is required for efficient in vivo spontaneous reactivation of HSV-1 from latency. *J Virol* **68:** 8045–8055.

Perng GC, Esmaili D, Slanina SM, Yukht A, Ghiasi H, Osorio N, Mott KR, Maguen B, Jin L, Nesburn AB, Wechsler SL (2001a). Three herpes simplex virus type 1 latency-associated transcript mutants with distinct and

asymmetric effects on virulence in mice compared with rabbits. *J Virol* **75:** 9018–9028.

Perng GC, Ghiasi H, Slanina SM, Nesburn AB, Wechsler SL (1996a). The spontaneous reactivation function of the herpes simplex virus type 1 LAT gene resides completely within the first 1.5 kilobases of the 8.3- kilobase primary transcript. *J Virol* **70:** 976–984.

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

Perng GC, Slanina SM, Ghiasi H, Nesburn AB, Wechsler SL (1996b). A 371-nucleotide region between the herpes simplex virus type 1 (HSV-1) LAT promoter and the 2-kilobase LAT is not essential for efficient spontaneous reactivation of latent HSV-1. *J Virol* **70:** 2014–2018.

Perng GC, Slanina SM, Ghiasi H, Nesburn AB, Wechsler SL (2001b). The effect of latency-associated transcript on the herpes simplex virus type 1 latency-reactivation phenotype is mouse strain-dependent. *J Gen Virol* **82:** 1117–1122.

Perng GC, Slanina SM, Yuhkt A, Ghiasi H, Nesburn AB, Wechsler SL (1999). Herpes simplex virus type 1 serum neutralizing antibody titers increase during latency in rabbits latently infected with LAT positive but not LAT negative viruses. *J Virol* **73:** 9669–9672.

Perng GC, Slanina SM, Yukht A, Ghiasi H, Nesburn AB, Wechsler SL (2000b). The latency-associated transcript gene enhances establishment of herpes simplex virus type 1 latency in rabbits. *J Virol* **74:** 1885–1891.

Rock DL, Nesburn AB, Ghiasi H, Ong J, Lewis TL, Lokensgard JR, Wechsler SL (1987). Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. *J Virol* **61:** 3820–3826.

Sawtell NM, Thompson RL (1992). Herpes simplex virus type 1 latency-associated transcription unit promotes anatomical site-dependent establishment and reactivation from latency. *J Virol* **66:** 2157–2169.

Steiner I, Spivack JG, Lirette RP, Brown SM, MacLean AR, Subak-Sharpe JH, Fraser NW (1989). Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. *EMBO J* **8:** 505–511.

Stevens JG, Wagner EK, Devi-Rao GB, Cook ML, Feldman LT (1987). RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science* **235:** 1056–1059.

Vilaplana L, O'Reilly DR (2003). Functional interaction between Cydia pomonella granulovirus IAP proteins. *Virus Res* **92:** 107–111.

Wald A, Zeh J, Selke S, Warren T, Ryncarz AJ, Ashley R, Krieger JN, Corey L (2000). Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *N Engl J Med* **342:** 844–850.

Whitley RJ (1996). Herpes simplex virus. In: *Field's virology*. Fields B, Knipe D, Howley P, Chanock R, Melnick J, Monath T (eds). Philadelphia: Lippincott-Raven, pp. 1843–1887.

Whitley RJ, Roizman B (2001). Herpes simplex virus infections. *Lancet* **357:** 1513–1518.

Xu F, Sternberg MR, Kottiri BJ, McQuillan GM, Lee FK, Nahmias AJ, Berman SM, Markowitz LE (2006). Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* **296:** 964–973.