Journal of NeuroVirology, 14: 558–562, 2008
© 2008 Journal of NeuroVirology
ISSN 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280802275912

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Short Communication

A speculated ribozyme site in the herpes simplex virus type 1 latency-associated transcript gene is not essential for a wild-type reactivation phenotype

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During herpes simplex virus-1 (HSV-1) latency in sensory neurons, LAT (latency-associated transcript) is the only abundantly expressed viral gene. LAT plays an important role in the HSV-1 latency-reactivation cycle, because LAT deletion mutants have a significantly decreased reactivation phenotype. Based solely on sequence analysis, it was speculated that LAT encodes a ribozyme that plays an important role in how LAT enhances the virus' reactivation phenotype. Because LAT ribozyme activity has never been reported, we decided to test the converse hypothesis, namely, that this region of LAT does not encode a ribozyme function important for LAT's ability to enhance the reactivation phenotype. We constructed a viral mutant (LAT-Rz) in which the speculated ribozyme consensus sequence was altered such that no ribozyme was encoded. We report here that LAT-Rz had a wild-type reactivation phenotype in mice, confirming the hypothesis that the speculated LAT ribozyme is not a dominant factor in stimulating the latency-reactivation cycle in mice. *Journal of NeuroVirology* (2008) **14,** 558–562.

Keywords: herpes simplex virus; LAT; latency; reactivation; ribozyme

Following primary ocular herpes simplex virus type 1 (HSV-1) infection, the virus established life long latency in neurons of the trigeminal ganglia (TG). Sporadic viral reactivation in the TG can lead to shedding of virus in tears, and less often to recurrent corneal disease, a significant cause of corneal blindness due to an infectious agent (Nesburn, 1983; Smith *et al,* 1980). During latency, latency-associated transcript (LAT) is the only abundant viral transcript (Rock *et al*, 1987; Stevens *et al*, 1987). LAT-null mutants have a significantly reduced reactivation phenotype (Hill *et al*, 1990; Leib *et al*, 1989, 1991; Perng *et al*, 1994; Sawtell and Thompson, 1992; Steiner *et al*, 1989, Trousdale *et al*, 1991). Thus, LAT provides a function that directly or indirectly enhances the HSV-1 reactivation phenotype.

Restoring expression of LAT nucleotides (nts) 1 to 1499 to an otherwise LAT-null mutant can restore the reactivation phenotype to wild-type levels (Perng *et al*, 1996). Thus there is a LAT function that resides completely within the first 1.5 kb of the primary 8.3-kb LAT transcript that is sufficient for supporting a wild-type reactivation phenotype. LAT has antiapoptosis activity (Ahmed *et al*, 2002; Carpenter *et al*, 2007; Kang *et al*, 2003; Perng *et al*, 2000) that appears to be a key factor in how LAT enhances the reactivation phenotype because (1) the region of LAT that can block apoptosis appears to co-map with the region of LAT (the first 1.5 kb of

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This work was supported by Public Health Service grants EY013191, EY018171, EY016663, 1P20RR15635, and R21AI069176, USDA grants 2005-01554 and 2006-01627, the Discovery Eye Foundation, the Skirball Program in Molecular Ophthalmology, and Research to Prevent Blindness. S.L.W. is an RPB Senior Scientific Investigator.
Received 1 April 2008; revised 7 May 2008; accepted 3 June 2008.

LAT) that enhances the reactivation phenotype (Inman *et al*, 2001); and (2) mutants in which the HSV-1 LAT gene is replaced by an alternative antiapoptosis gene have a wild-type reactivation phenotype (Jin *et al*, 2005, 2007; Mott *et al*, 2003; Perng *et al*, 2002).

Theoretically, LAT's function could be due to its genomic DNA, a LAT RNA, and/or a LAT protein. Apart from the promoter driving high levels of LAT expression, a direct DNA function is highly unlikely because plasmids or viral mutants containing just the LAT promoter or just the LAT structural region without the LAT promoter do not block apoptosis and do not have a wild-type reactivation phenotype (Hill *et al*, 1990; Inman *et al*, 2001; Jin *et al*, 2003; Perng *et al*, 1999, 2000). Thus, LAT appears to function either via one or more protein intermediaries, via one or more RNA functions, or combinations of proteins and RNAs. The search for a LAT protein has been long and extensive. Although there is a recent report of a LAT-encoded protein (Thomas *et al*, 2002), this putative protein maps completely outside of the functional first 1.5 kb of LAT, suggesting that it is not critical for LAT's ability to enhance the reactivation phenotype. The first 1.5 kb of LAT contains eight potential open reading frames (ORFs), none of which are well conserved among HSV-1 LAT genes from different HSV-1 strains, suggesting that none of them are critical to LAT's function (Drolet *et al*, 1998). In contrast, changing all eight initiating ATGs to TTGs in a plasmid expressing just the first 1.5 kb of LAT completely abrogated the LAT plasmid's antiapoptosis activity (Carpenter *et al*, 2008). Although this suggests that one or more of these potential LAT proteins is involved in blocking apoptosis, it is also possible that introducing these point mutations altered the LAT RNA structure or stability thus altering an undetermined LAT RNA activity.

Recently a micro-RNA (miRNA) was reported to be encoded upstream of the minimal LAT promoter (Cui *et al*, 2006), and interest has been raised regarding the possibility that LAT may encode one or more functional miRNAs. Consistent with this, we have recently found two small RNAs that are encoded from within the first 1.5 kb of LAT (Peng *et al*, 2008). It has not yet been determined whether these small LAT RNAs are miRNAs or some other type of small RNA. Nor has it been determined whether they are involved in LAT's antiapoptosis activity or LAT's ability to enhance the reactivation phenotype.

One type of small RNA that functions without encoding a functional protein is a ribozyme. The consensus sequence ''GAA(G/A)C'' is required for ribozyme activity (Hui and Lo, 1998). LAT contains only one such sequence ''GAAGC'' at LAT nts 1245 to 1249 (Hui and Lo, 1998). The authors who reported this hypothesized that LAT's function was dependent on this proposed ribozyme. Although this speculated ribozyme sequence is within the first 1.5 kb of LAT, and hence within the important functional region of LAT, we are not aware of any follow-up reports in over 10 years. We therefore hypothesized that if a ribozyme is encoded by this region of LAT, it probably does not play an important role in LAT's ability to enhance the reactivation phenotype. It would be difficult to demonstrate that LAT encodes a functional ribozyme without knowing what function to examine. Without a known function, it would be even more difficult to disprove the existence of a functional LAT ribozyme. We therefore reasoned that the most direct and powerful approach to test the hypothesis that the speculated LAT ribozyme is *not* critical for the LAT function(s) that enhance the reactivation phenotype would be to make a viral mutant in which the speculated LAT ribozyme sequence is disrupted in both copies of LAT. We report here that reactivation of the resulting ribozyme negative LAT mutant (LAT-Rz) in the mouse explant TG reactivation model was indistinguishable from that of wild-type virus. Thus, the speculated ribozyme is not essential for LAT's ability to enhance the HSV-1 reactivation phenotype in mice.

The consensus ribozyme sequence ''GAAGC'' at LAT nts 1245 to 1249 was changed to ''TAAGC'' using site directed mutagenic primers and a two-step polymerase chain reaction (PCR) reaction. Two PCR fragments were generated that overlap by 35 bp of identical sequence. This overlap contains the above alteration to the ribozyme consensus sequence. The two fragments were mixed in equal amounts and joined together by PCR. The resulting 445 bp long fragment contained a *Kpn*I restriction site at the 5' end and an *Xcm*I restriction site at the 3' end, with the mutated ribozyme consensus sequence in between. The fragment was cloned into the *Kpn*I/*Xcm*I restriction sites of a cloned *Hpa*I/*Sal*I fragment of McKrae DNA that overlaps the putative ribozyme site by over 800 bp on both ends. The mutated 445-bp region was then sequenced to confirm that the *Hpa*I/*Sal*I fragment contained the mutated ribozyme consensus site. This plasmid was designated LAT3.9Rz (Figure 1).

The LAT3.9Rz plasmid was then used to restore the entire LAT region from LAT nts −161 to +1667 in both copies of the LAT region of the LAT(−) mutant dLAT2903 (Figure 1). This was achieved by homologous recombination following cotransfection of RS cells with the plasmid and purified genomic dLAT2903 DNA. Viral plaques were screened with a *Sty*I-*Sty*I probe (LAT nts 76 to 447) for reintroduction of the LAT region into the dLAT2903 virus, as we previously described for construction of other LAT mutants (Jin *et al*, 2005; Perng *et al*, 1994). Following nine rounds of plaque purification, a viral stock was grown and subjected to Southern blot analysis to confirm that both copies of LAT had been restored to dLAT2903 by the mutated LAT region

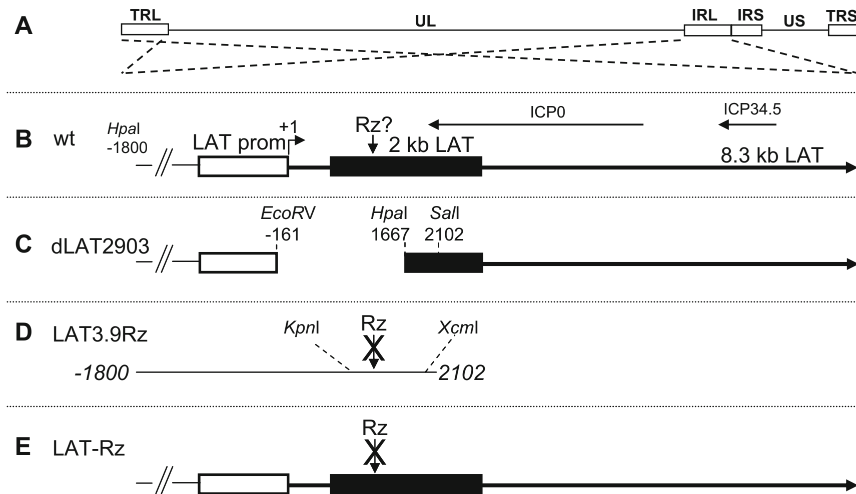

Figure 1 Structure and construction of the LAT-Rz mutant. (**A**) Genomic structure of wt HSV-1. The HSV-1 genome contains a long unique region (UL) bounded by inverted long repeats (TLR, terminal repeat long; IRL, internal repeat long) and a short unique region (US) bounded by inverted short repeats (IRS, internal repeat short; TRS, terminal repeat short). The LAT locus is located in the long repeats and is therefore present in two copies/genome. The crossing dashed lines indicate that the expanded view of the LAT regions in the panels below represents both LAT copies in opposite orientations. (**B**) Expanded view of the LAT region and some of its transcripts. The start of LAT transcription is at +1. The primary LAT transcript is 8.3 kb. The solid rectangle shows the relative location of the stable 2-kb LAT intron. The open rectangle indicates the LAT promoter region. The relative locations of the ICP0 and ICP34.5 mRNAs are shown. ''Rz?'' indicates the relative location of the putative LAT ribozyme site. (**C**) LAT-null mutant dLAT2903. dLAT2903 contains a deletion from an *EcoR*V restriction site at −161 relative to the start of LAT transcription (thus deleting essential elements of the LAT promoter) to a HpaI site at LAT nt 1667. It makes no detectable LAT RNA and has a significantly reduced reactivation phenotype. (**D**) Ribozyme-negative restriction fragment. The putative LAT ribozyme site in LAT3.9Rz at LAT nts 1245 to 1249 was changed from ''GAAGC'' to ''TAAGC'' using site directed mutagenic primers and a two-step PCR reaction and then cloning the resulting PCR product into the *Kpn*I/*Xcm*I restriction sites of a plasmid containing a *Hpa*I/*Sal*I fragment of wild-type HSV-1 McKrae DNA corresponding to LAT nts −1800 to +2102. The LAT3.9Rz sequence is identical to the McKrae sequence in this region except for the single bp change that eliminates the putative ribozyme site. (**E**) LAT ribozyme-negative mutant LAT-Rz. The LAT3.9Rz plasmid (**D**) was cotransfected into RS cells with dLAT2903 genomic DNA (**C**) to allow homologous recombination to restore the deleted region of dLAT2903 with LAT3.9Rz DNA, thus ''marker rescuing'' dLAT2903 with DNA containing the disrupted putative ribozyme site.

(Figure 2). DNA was digested with *Sal*I and the DNA fragments separated on a 0.8% agarose gel, denatured, and transferred to a nylon membrane for Southern blot analysis. A *Hpa*I-*Hpa*I DNA fragment (LAT nts −1800 to +1499) was radiolabeled with α-^{32}P-dCTP by nick translation and used as a probe. *Sal*I digestion produces a LAT DNA fragment from LAT in the terminal long repeat that is larger than the LAT DNA fragment from the internal long repeat, thus allowing us to look at the status of both copies of LAT. The enzyme cuts both copies of LAT at LAT nt 2102 and then cuts in the unique long region. The *Sal*I site in the unique long region near the terminal long repeat is 8768 nts away from LAT nt 2102 in the terminal long repeat, whereas the *Sal*I site in the unique long region near the internal long repeat is 6387 nts away from LAT nt 2102 in the internal long repeat. Thus, as seen in Figure 2, the wild-type LAT fragments are 8768 and 6387 bp, whereas the dLAT2903 LAT fragments are each 1829 nts shorter (6939 and 4558 bp) due to the *EcoR*V (−161) to *Hpa*I (1667) deletion in both copies of LAT. As shown in lane ''Rz,'' homologous recombination with the plasmid containing the mutated ribozyme site restored both LAT regions to their wild-type mobility, indicating that the viral mutant, LAT-Rz, contains a mutation in the putative LAT ribozyme sequence in both copies of LAT. This mutation renders both LAT regions incapable of encoding a functional ribozyme.

Replication of LAT-Rz in tissue culture was similar to replication of wild-type virus (not shown). To determine the effect of the putative LAT ribozyme knockout on the HSV-1 reactivation phenotype, Swiss-Webster mice were infected with 2×10^5 plaque-forming units (PFU)/eye of LAT-Rz, its immediate parental virus the LAT deletion mutant dLAT2903, or HSV-1 strain McKrae, the parental wild-type virus from which dLAT2903 was constructed. Acute eye disease (not shown) and survival (58% to 72%, $P > .05$) were similar with all three viruses.

Mouse TG explant reactivation was performed as previously described (Perng *et al*, 2001). Briefly, 30 days post infection mice were euthanized, TG were removed, and individual TG, each cut into approximately 10 to 12 pieces, were plated in 6-well tissue culture plates in standard tissue culture medium (MEM) with 10% fetal calf serum (FCS). An aliquot of medium was removed daily from each well and plated onto monolayers of rabbit skin cells, which were then monitored for the appearance of cytopathic effects (CPEs) indicative of the presence of reactivated virus. The time of first appearance of

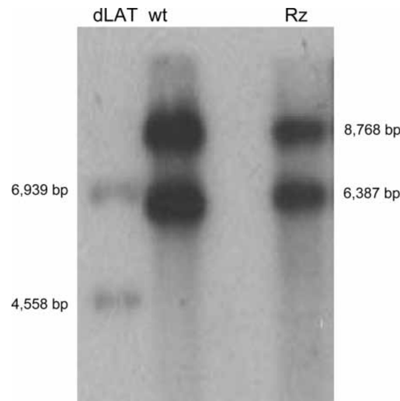

Figure 2 Southern blot of LAT-Rz genomic DNA. Genomic DNA was prepared from dLAT2903 (the immediate parent virus of LAT-Rz), wild-type McKrae (the immediate parent virus of dLAT2903), and LAT-Rz. The DNAs were digested with *Sal*I and Southern analysis performed using a *Hpa*I-*Hpa*I (LAT nts −1800 to +1499) probe as described in the text. *Sal*I digestion cuts at LAT nt 2102 and in the unique long region producing a larger LAT containing fragment from the terminal long repeat compared to the internal long repeat (see text). The wild-type LAT fragments are 8768 and 6387 bp, whereas the dLAT2903 LAT fragments are each 1829 nts shorter (6939 and 4558 bp) due to the *EcoR*V to *Hpa*I deletion in both LAT genes. Lanes: dLAT, dLAT2903; wt, wild-type HSV-1 McKrae; Rz, LAT-Rz. There is a blank lane between wt and Rz.

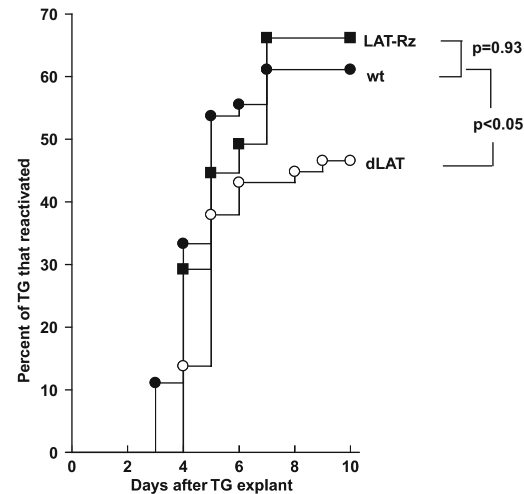

Figure 3 Reactivation of LAT-Rz. Swiss Webster mice were ocularly infected and individual TG removed 30 days p.i. for explant reactivation analysis as described in the text. The days at which reactivated virus first appeared in the TG cultures were plotted as a survival curve. LAT-Rz, $n = 65$ TG; dLAT2903, $n = 58$ TG; wild type, $n = 54$ TG.

reactivated virus in the TG explant cultures is shown in Figure 3. Consistent with previous studies (Perng *et al*, 1994, 2001), reactivation of dLAT2903 was significantly reduced compared to wild-type McKrae ($P = .046$ by survival curve analysis). Reactivation of LAT-Rz was indistinguishable from wild-type virus ($P = .93$) and was significantly greater than dLAT2903 ($P = .034$). Thus, the putative LAT ribozyme did not appear to be essential for LAT's ability to support the wild-type reactivation phenotype.

The speculated LAT ribozyme site mutated in this report was noted almost 10 years ago based on sequence analysis of the LAT region (Hui and Lo, 1998). At that time, it was proposed that LAT encoded a functional ribozyme that plays a significant role in how LAT affects the reactivation phenotype. The proposed LAT ribozyme site is located within a larger region of LAT that is involved in LAT's ability to enhance the virus' reactivation phenotype (Perng *et al*, 1994, 1996). However, because these mutants affect a much larger region than the small potential ribozyme site, they do not demonstrate that this ribozyme site is important as was previously suggested (Hui and Lo, 1998). To our knowledge, the LAT-Rz mutant in this report is the only mutant that directly targets the potential ribozyme site. Because the predicted LAT ribozyme site has been abrogated in LAT-Rz without any detectable alteration to the HSV-1 reactivation phenotype, it appears that the predicted ribozyme is not essential for LAT's ability to enhance the HSV-1 reactivation phenotype in mice.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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.

Carpenter D, Henderson G, Hsiang C, Osorio N, Benmohamed L, Jones C, Wechsler S L (2008). Introducing point mutations into the ATGs of the putative open reading frames of the HSV-1 gene encoding the latency associated transcript (LAT) reduces its anti-apoptosis activity. *Microb Pathog* **44:** 98–102.

Carpenter D, Hsiang C, Brown DJ, Jin L, Osorio N, BenMohamed L, Jones C, Wechsler SL (2007). Stable cell lines expressing high levels of the herpes simplex virus type 1 LAT are refractory to caspase 3 activation and DNA laddering following cold shock induced apoptosis. *Virology* **369:** 12–18.

Cui C, Griffiths A, Li G, Silva L M, Kramer MF, Gaasterland T, Wang XJ, Coen DM (2006). Prediction and identification of herpes simplex virus 1-encoded microRNAs. *J Virol* **80:** 5499–4508.

Drolet BS, Perng GC, Cohen J, Slanina SM, Yukht A, Nesburn A B, 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.

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.

Hui EK, Lo SJ (1998). Does the latency associated transcript (LAT) of herpes simplex virus (HSV) func-

tion as a ribozyme during viral reactivation?. *Virus Genes* **16:** 147–148.
Inman M, Perng G, Henderson G, Ghiasi H, Nesburn A, Wechsler S, Jones C (2001). 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, Carpenter D, Mott KR, Osorio N, Naito J, Brick D J, Jones C, Wechsler SL (2007). Reactivation phenotype in rabbits of a herpes simplex virus type 1 mutant containing an unrelated antiapoptosis gene in place of latency-associated transcript. *J NeuroVirol* **13:** 78–84.
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.
Kang W, Mukerjee R, Fraser NW (2003). Establishment and maintenance of HSV latent infection is mediated through correct splicing of the LAT primary transcript. *Virology* **312:** 233–244.
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.
Leib DA, Nadeau KC, Rundle SA, Schaffer PA (1991). The promoter of the latency-associated transcripts of herpes simplex virus type 1 contains a functional cAMP-response element: role of the latency-associated transcripts and cAMP in reactivation of viral latency. *Proc Natl Acad Sci U S A* **88:** 48–52.
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.
Nesburn AB (1983). *Report of the Corneal Disease Panel: Vision research: a national plan 1983–1987*. St. Louis: C.V. Mosby.
Peng, W, Vitvitskaia, O, Carpenter, D, Wechsler, S. L. & Jones, C. (2008). Identification of two small RNAs within the first 1.5-kb of the herpes simplex virus type 1 (HSV-1) encoded latency-associated transcript (LAT). *J NeuroViol* **14**: 41–52.
Perng G, Jones C, Ciacci-Zanella H, Henderson G, Yukht A, Slanina S, Hofman F, Ghiasi H, Nesburn A, Wechsler S (2000). 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, Ghiasi H, Slanina SM, Nesburn AB, Wechsler SL (1996). 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 (2001). 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, Drolet BS, Keleher WJ, Ghiasi H, Nesburn AB, Wechsler SL (1999). A herpes simplex virus type 1 latency associated transcript (LAT) mutant with increased virulence and reduced spontaneous reactivation. J. *Virol.* **73:** 920–929.
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.
Smith RE, McDonald HR, Nesburn AB, Minckler DS (1980). Penetrating keratoplasty: changing indications, 1947 to 1978. *Arch Ophthalmol* **98:** 1226–1229.
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.
Thomas SK, Lilley CE, Latchman DS, Coffin RS (2002). A protein encoded by the herpes simplex virus (HSV) type 1 2-kilobase latency-associated transcript is phosphorylated, localized to the nucleus, and overcomes the repression of expression from exogenous promoters when inserted into the quiescent HSV genome. *J Virol* **76:** 4056–4067.
Trousdale MD, Steiner I, Spivack JG, Deshmane SL, Brown SM, MacLean AR, Subak-Sharpe JH, Fraser NW (1991). In vivo and in vitro reactivation impairment of a herpes simplex virus type 1 latency-associated transcript variant in a rabbit eye model. *J Virol* **65:** 6989–6993.

This paper was first published online on iFirst on 3 November 2008.