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Review

The latency associated transcripts (LAT) of herpes simplex virus: still no end in sight

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The herpes simplex virus latency associated transcripts (LAT) are the only viral gene products that accumulate to a high concentration in the trigeminal ganglia (TG) of latently infected animals. Their abundance is particularly surprising, since they are thought to be the introns of a larger, ~8.3 kb precursor. LAT are not linear molecules. Therefore they are either a circle or a lariat that is not debranched. This structure could explain their unusual high stability. Moreover, the functional potential of stable, nuclear RNA has been demonstrated in other biological systems and could offer a clue as to the mechanism of action of LAT. Therefore, the non-linear nature of LAT and functional implications mean that both literally and figuratively, there is no end in sight for this unusual molecule.

Keywords: HSV neuronal latency; latency associated-transcripts (LAT); RNA physical structure; viral reactivation

Introduction

Herpesviruses are ubiquitous and have been isolated from species as diverse as channel catfish and primates (Roizman, 1996a). One unifying feature of all members of this family is their ability to establish latent infections in their natural hosts (Stevens and Cook, 1971; Stevens, 1994). There are at least eight subfamily members whose natural hosts are humans, and these can be distinguished, in part, by the tissue site in which they establish latency (Table 1). Latent infections can be clinically defined as the presence of viral genetic information in the absence of clinical infection (Fraser *et al*, 1992). Also, all latent tissue has no infectious virus in cell free extracts. From the molecular perspective, latency can be defined as the presence of the entire viral genome in a setting of very limited, if any, viral gene expression. Since the lymphotropic and neurotropic herpesviruses form latent infections in dividing and non-dividing cells, respectively (see Table 1), one could reasonably assume that distinct molecular strategies are employed. For example, the molecular mechanisms governing Epstein Barr virus (EBV) latent infection of B lymphocytes appear to involve continuous expression of a variety of different viral genes (Kieff, 1996) and the use of an origin of replication distinct from that used during the productive phase (Stevens, 1994). For the neurotropic herpesviruses, the best studied example is that of human herpes simplex virus (HSV). Because even experimental viral infections of mice and rats result in HSV latency of 5–30% of the 10 000 terminally differentiated neurons (Mehta *et al*, 1994; Mitchell *et al*, 1994; Ramakrishnan *et al*, 1994), and reproduction of latent infection *in vitro* has been troublesome, an understanding of the molecular details of HSV latency has been elusive. The realization that in neurons latently infected with HSV there is a major class of viral gene products called latency associated transcripts (LAT), was an exciting discovery that held the promise that the key to latency would be revealed. These transcripts can be detected by either conventional *in situ* or Northern blot hybridization (Stevens *et al*, 1987; Deatly *et al*, 1987). Since the LAT were first described 10 years ago, PCR studies suggest that they are not the only viral gene transcripts in latently infected tissue (Kosz-Vnenchak *et al*, 1993; Kramer and Coen, 1995). These rare viral transcripts could be present as a normal component of latency or the result of a very small number of cells undergoing reactivation. There are many (perhaps a majority) latently infected cells that do not express detectable concentration of LAT (Mehta *et al*, 1994, 1995;

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Mitchell, 1994; Maggioncalda *et al*, 1996). The ratio of latent cells to LAT containing cells has been estimated to be between 2 : 1 and 4 : 1 (Maggioncalda *et al*, 1996; Mehta *et al*, 1994, 1995; Mitchell, 1994; Ramaknishnan *et al*, 1994).

The LAT could have no biological role and could be a 'selfish' RNA in the sense that the RNA accumulates with no apparent purpose other than its own survival. However, the fact that they are so distinctly abundant, at least in a subpopulation of latently infected cells, makes speculation about their nature and function irresistible. Accumulation of a high concentration of a specific RNA suggests an important and meaningful role in latency and reactivation. Since they are lariats i.e., lacking two ends, there is no end in sight for them, both physically and as a topic of discussion. Hence, investigations of LAT continue, and the question remains as to whether the LAT are important because they are abundant, or abundant because they are important.

LAT domain – important landmarks

Figure 1 describes the LAT domain encompassing the very beginning of the LAT promoter at the 5′ end, the CAP site, the beginning of the 2.0 stable LAT intron, and the extreme 3′ end of the transcript. Table 2 gives the genomic sites of specific elements. Viruses with deletion mutations have been constructed in numerous regions of the LAT domain. The sites of these deletions as well as certain trancriptional activation sites are noted in Figure 1. There are also numerous other transcriptional activating sites in the LAT domain. These have been reviewed in other publications (Roizman and Sears, 1996b; Wagner, 1994; Wagner *et al*, 1995).

Table 1 Human herpesviruses and the tissue in which they establish latency

Sub-family name	*Tissue in which latency is established*[a]
Herpes simplex virus type 1	Peripheral and central nervous system
Herpes simplex virus type 2	Peripheral and central nervous system
Varicella Zoster Virus	Peripheral and central nervous system
Epstein Barr Virus	B lymphocytes
Cytomegalovirus	Lymphocytes and certain other tissues
Human Herpesvirus 6	Peripheral blood mononuclear cells
Human Herpesvirus 7	Lymphocytes ($CD4^+$)
Human Herpesvirus 8 (Kaposi's sarcoma associated herpes virus)	Endothelial cells, invading lymphocytes

[a]See: Katsafanas *et al* (1996); Kieff (1996); Roizman (1996a); Roizman and Sears (1996b); Straus (1993)

17ΔPst, was the first viral construct that was a LAT-expression negative mutant, was shown to have significantly reduced spontaneous and induced frequencies of ocular reactivation in the

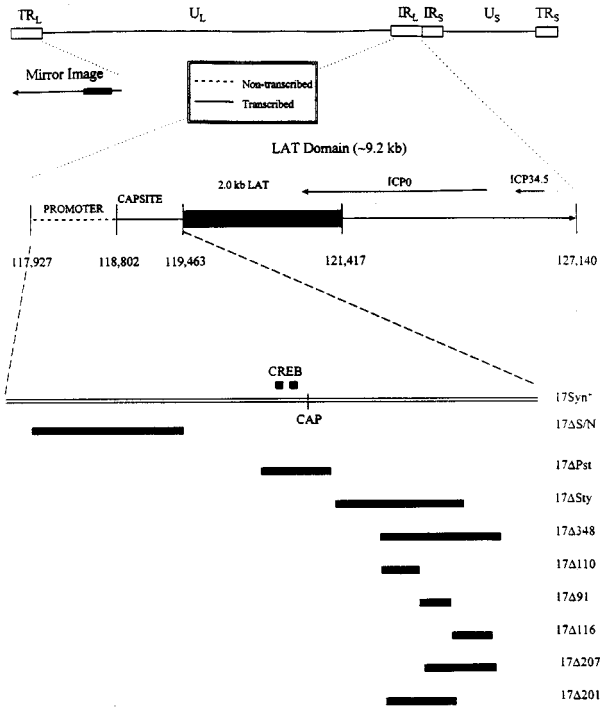

Figure 1 LAT domain – important landmarks. The region encoding the LAT domain is shown with genomic sites numbered according to $17Syn^+$ parent sequence as published in the GenBank revision of 25 November 1995. Thick lines, major 2.0 kb LAT; dashed lines, LAT promoter region. Arrows indicate the direction of transcription. Locations of deletions in relevant viral constructs are given below the parent sequence. The bottom part of the Figure noting each deletion is drawn to scale. Two cyclic AMP response element binding (CREB) sites are noted. Labeled genomic sites: TR_L terminal long repeat; U_L unique long; IR_L, internal repeat long; IR_S internal repeat short; U_S, unique short; TR_S, terminal repeat short.

Table 2 Location of selected elements in the LAT domain

Element	*Positions*	*Size*
LAT domain	117927–127140	9186 bp (9.2 kb)
ICPO	120672–124249	3573bp (3.6 kb)
ICP34.5	125110–115859	749 bp
LAT promoter	117927–118802	875 bp
CAP site to LAT 3′ end	118802–127140	8338 bp (8.3 kb)
CAP site to LAT 5′ intron	118802–119463	661 bp
LAT intron	119463–121417	1954 bp (2.0 kb)
17ΔS/N deletion	118006–118443	437 bp
17ΔPst deletion	118664–118866	202 bp
17ΔSty deletion	118880–119350	370 bp
17Δ348 deletion	119007–119355	348 bp
CREB-#1	118759–118765	7 bp
CREB-#2	118717–119724	8 bp
TATA box	118772–118776	4 bp

rabbit (Bloom *et al*, 1994). Other deletion mutants (Block *et al*, 1993; Devi-Rao *et al*, 1994; Hill *et al*, 1990; Ho and Mocarski, 1989; Steiner *et al*, 1989; Trousdale *et al*, 1991; Perng *et al*, 1994) that are LAT negative viruses have also been shown to reactivate significantly less frequently. These viruses cause acute infections similar to that of their parent strain. The parent for most of these viruses is 17Syn$^+$. HSV-1 strain McKrae, which has a phenotype almost identical to 17Syn$^+$ has also been used (Perng *et al*, 1994, 1996a,b). Marker rescued viruses have been constructed from many of these mutant viral constructs. The rescued viruses exhibited the same growth and reactivation rates as the parent.

Two deletion viruses, 17ΔSty (a 370 bp deletion) and 17Δ348 (a 348 bp deletion), were shown to have significantly reduced adrenergically-induced ocular reactivation in the rabbit eye model (Bloom *et al*, 1996; Hill *et al*, 1996b). Therefore, these regions are essential for maximal adrenergic reactivation. These two viruses were constructed using different techniques and have approximately 70% overlap in the base pair sequences of the deleted regions (see Figure 1). The rescued viruses of both of these deletion viruses displayed normal acute pathology and reactivated as frequently as the parent, 17Syn$^+$. On the other hand, Perng *et al* (1996b) used a virus with a 371 bp deletion nearly identical to 17ΔSty and reported normal frequency of spontaneous HSV-1 ocular shedding in the rabbit eye model. This suggests that the genomic region controlling spontaneous reactivation could be separate and distinct from that controlling adrenergically induced reactivation.

We (Bloom *et al*, 1996; Hill *et al*, 1996b, 1997; Wu *et al*, 1996) have suggested that methylation within the LAT region could be one possible mechanism controlling reactivation. The deleted sequences from 17ΔSty and 17Δ348 are high in their GC content and also have a high ratio of CpG : GpC dinucleotides. These characteristics are indicative of increased potential for cytosine methylation in this region. Bloom (1997) and Su *et al* (1997) have detected methylation in this region. We hypothesize that this could be a mechanism for regulating and controlling reactivation and recurrent disease.

Taken together, the results from 17Δ348, 17ΔSty, and 17ΔPst suggest that sequences as small as 200 bp located somewhere between 118 880 and 119 355 (Table 1 and Figure 1) are needed for adrenergically induced reactivation in the rabbit. Site-specific mutations of potential start codons in the 17Δ348 region, as well as a series of small, nonoverlapping deletions ranging from 91–116 base pairs within this 348 bp region have all reactivated following epinephrine iontophoresis with the same frequency as the parent in the rabbit eye. If the exact region identified by deletion analysis (348 bp) is required for adrenergic reactivation, it is surprising that these site-directed mutations are phenotypically normal, since their deletions are in this essential domain. This presents a possible dilemma in our hypothesis that one of these three smaller deletion viruses, which completely comprised as individual components the 348 bp deletion, would not reactivate (Bloom *et al*, 1996). Our current hypothesis is that the normal reactivation frequencies observed with these small deletion viruses is due to the sequence size or configuration, the degree of methylation, or co-operative factors in two or more of these regions is necessary for adrenergic HSV reactivation. Ongoing experiments involving deletion of methylation and creation of larger, overlapping viral constructs within the 348 bp region (i.e. 17Δ201 and 17Δ207, see Figure 1) will perhaps determine whether a very specific sequence in the 348 bp region is critical for reactivation.

A seven base pair mutation in the cyclic AMP response element binding (CREB) site closest to the TATA box resulted in a significant reduction in reactivation frequency following epinephrine iontophoresis (Bloom *et al*, 1997). The rescued virus and the parent, 17Syn$^+$, reactivated with high frequency. This suggests a specific adrenergically-induced response mediated by this activator.

We have recently found that a 437 bp sequence at the beginning of the LAT promoter is essential for *in vivo* reactivation in the rabbit eye model (Hill *et al*, 1997). This deletion is located between genomic positions 118 006 and 118 443. This virus, designated 17ΔS/N, has been analyzed in both rabbit and mouse models (Hill *et al*, 1997; Maggioncalda *et al*, 1994). In the rabbit reactivation model, this region was shown to be essential for induced reactivation. However, in the mouse model, co-cultivation of the ganglia demonstrated that the rate and frequency of the 17ΔS/N was the same as that of the parent and the rescuant. The co-cultitvation data in the rabbit was the same as in the mouse. No *in vivo* reactivation has been done in the mouse with 17ΔS/N virus. Since the deletion virus does not synthesize a 1.1 and 1.8 kb transcript, the results suggest that these molecules are involved in the control of *in vivo* reactivation.

Lots of LAT

LAT are detected as a 2.0 kb species in productive infection and during latency as both 1.45/1.50 kb and 2.0 kb species (Wagner, 1994; Wagner *et al*., 1995). They are plentiful, with a single latently infected neuron containing at least 40 000 copies (Wagner *et al*, 1988) and as many as 100 000 copies per cell. For the most part, LAT are restricted to the nucleus and lack polyadenylation. As shown in Figure 2, the 1.45–2.0 kb LAT have been hypothesized to be stable introns, being derived from the processing of a large 8.3 kb precursor (Devi-Rao *et*

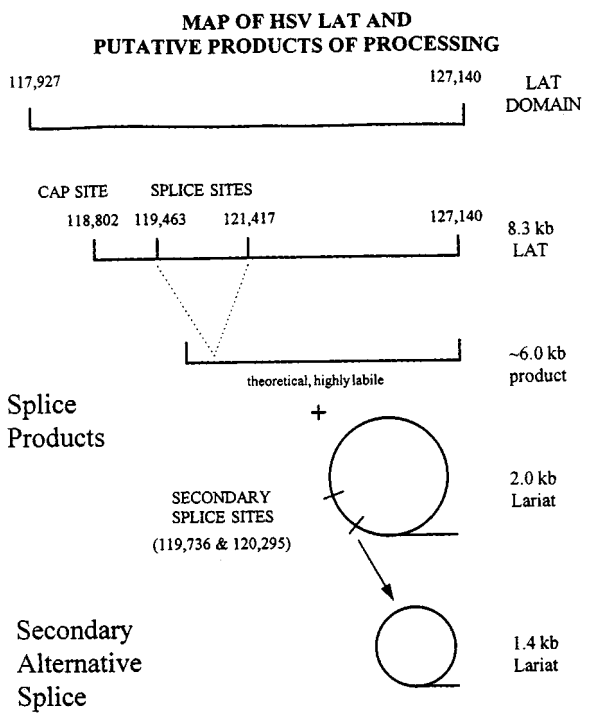

Figure 2 Map of HSV LAT and putative products of processing. The primary LAT transcript, predicted to be approximately 8.3 kb, is shown, with genomic locations of nucleotide sequences marked with reference to the entire HSV-1 genome. The primary transcript is hypothesized to be spliced into a ~6 kb product, with 1.96 and 1.45 kb introns also generated. The 1.45 kb intron could be derived directly from the 8.3 kb and not from the 2.0 kb LAT. Lariat representations of LAT are shown.

al, 1991; Farrell *et al*, 1991). The precursor and putative 6.0 kb splice product have been difficult to detect.

Nonlinearity of the 1.45 and 2.0 kb LAT

If LAT are introns, as suggested by Farrell *et al* (1991), their stability is all the more surprising. Wu *et al* (1996) and Rodahl and Haarr (1997) have provided direct evidence that the LAT were non-linear. Wu *et al* (1996) used HSV-1 strain 17Syn$^+$ in mouse trigeminal ganglia. Rodahl and Haarr (1997) used HSV-1 strain KOS (M), in PC12 cells. Wu *et al* (1996) show that the putative 2.0 kb LAT migrated in agarose gels significantly more slowly (at 2.25 kb), even under denaturing conditions, than would be expected for a species of its primary sequence length of 1954 nucleotides. This implied a significant secondary structure, possibly circular. The circular nature of the major LAT was demonstrated by converting the 2.25 kb species, derived from both latently and productively infected cells, into a linear 1.960 kb form using both chemical and enzymatic methods (Figure 3). The cumulative evidence suggests that both the 1.45 and 2.0 kb

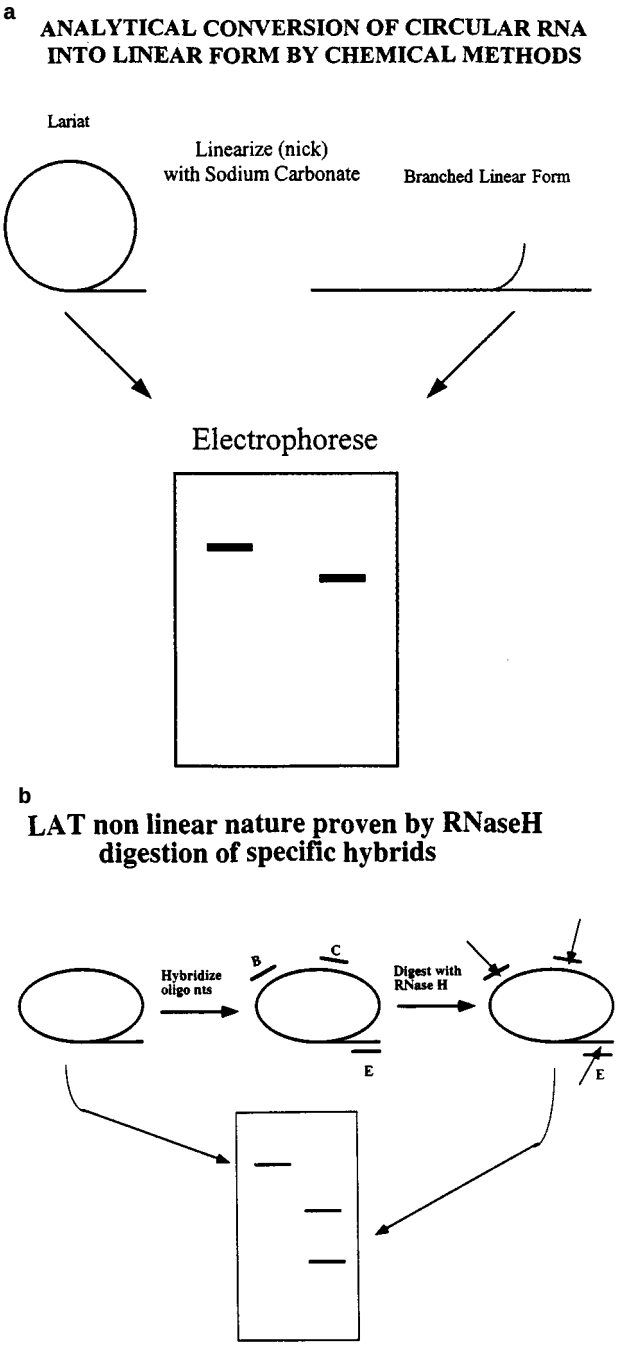

Figure 3 Analytical conversion of circular RNA into linear forms by chemical and enzymatic methods. The strategy used by Wu *et al* (1996a) to prove the circular (lariat) nature of HSV LAT is shown. LAT is represented as a lariat/loop structure that would migrate in agarose gels (rectangular box) more slowly than a linear molecule of the same molecular weight and sequence composition. The putative physical forms of the LAT are shown before and after linearization (**a**) or hybridization to complementary oligonucleotides (**b**). Products are resolved by agarose gel electrophoresis (rectangular boxes). Conversion of the circular (lariat) LAT into the linear form by chemical (**a**) or enzymatic (**b**) processes results in faster migrating molecules. In (**b**), LAT is digested with RNase H, which recognizes and cleaves RNA/DNA hybrids, after hybridization to short complementary oligonucleotides. Note that only two products are resolved, since oligo 'E' hybridizes to the short single stranded lariat tail.

LAT are lariats, rather than true circles. This evidence includes (i) oligonucleotide primers that would have directed synthesis of PCR products of an 'endless' RNA substrate were unsuccessful; (ii) single stranded tails could be detected by hybridization and RNase cleavage analysis, and (iii) circular molecules remained even after digestion with RNaseH, if oligonucleotides complementary to the putative single stranded tail were used (see Figure 3b). Rodahl and Haar (1997) obtained very similar results in PC12 cells. The lariat nature is further supported by data showing that replacement of the putative branchpoint with a 'consensus' branchpoint sequence results in a LAT that does not accumulate, presumably due to debranching (Wu *et al*, 1996).

The branchpoint of LAT

If the latently associated transcripts are stable lariats, the detection of the branchpoint should be possible by physical and genetic means. Wu *et al* (1996) speculated that the nucleotide sequence beginning at 121 391 (GAGGGAG) could serve as the branchpoint, since it has many properties consistent with the consensus sequences (Figure 4) and is followed by a pyrimidine rich region. This sequence departs significantly from the consensus branchpoint sequence and is predicted to be responsible for the accumulation of LAT lariats (Wu *et al*, 1996). Experiments are underway to test this hypothesis. Thus, the non-consensus nature of this branchpoint is probably the explanation for its relative fraction to host debranching enzymes and could be the key to LAT stability. Indeed, introduction of such a branchpoint into other RNA sequences could be useful in the production of stable RNA molecules.

Generation of circular RNA and the nature of the lariat

Circular RNA is more stable than linear forms. The genome and anti-genome of hepatitis delta virus are stable circular RNA molecules, resulting from the unusual ribozyme-mediated processing activity that is intrinsic to the viral RNA nucleic acid sequences (Lazinsky and Taylor, 1995). Other examples of circular RNA molecules have recently been described in mammalian systems (Bailleul, 1996; Capel *et al*, 1993). These could result from missplicing (causing exon scrambling) in which the pre-mRNA assumes a loop structure and non-sequential exons are spliced together (Zaphiropoulos, 1996). Curiously, unlike the LAT, the circular RNA product of cytochrome P450 transcript missplicing has been found predominantly in the cytoplasm (Zaphiropoulos, 1996).

Lariat structures are normal transition intermediates of group II type intron splicing reactions. Lariats (see Figure 4 are structures with only one terminus; containing a 2′ hydroxol esterified to a 5′ phosphate group. After splicing, most introns are rapidly degraded. The few known examples of lariats that accumulate, do so in the nucleus (Qian *et al*, 1992). The half life of a typical lariat is measured in minutes, not days as is HSV LAT (Bailleul, 1996; Rodahl and Haarr, 1997; Zabololny *et al*, 1997). If these transcripts are stable lariats, as we postulate, the explanations for their stability could be (i) the inherent physical resistance to debranching, (ii) the induction of functions that interfere with host debranching, or (iii) lack of debranching activity in cells accumulating LAT. Although possibilities (ii) and (iii) have not been ruled out, the ability of plasmids expressing only LAT (and no other HSV functions) to induce stable LAT lariats suggests that no other HSV functions are necessary for accumulation of stable LAT (Farrell *et al*, 1991; Su *et al*, 1997; Wu *et al*, 1997).

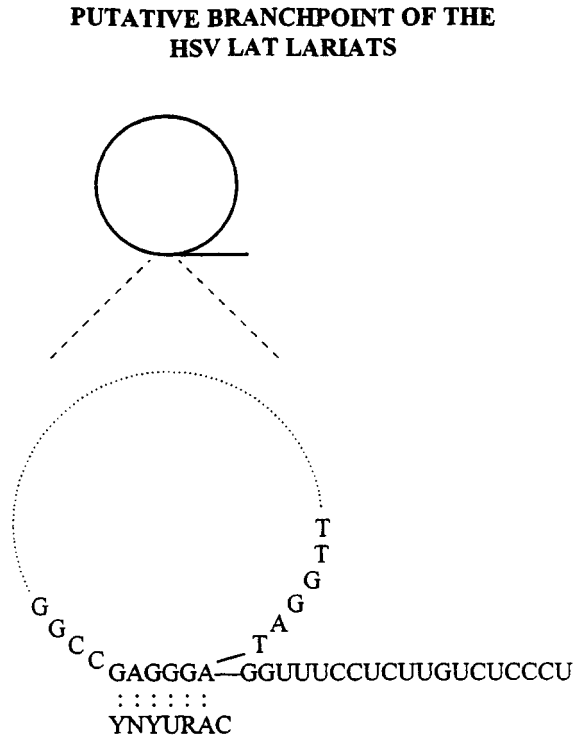

Figure 4 Putative branchpoint of the HSV LAT lariats. The putative LAT lariat is shown with the sequence between nucleotides 121 381 to 121 412 presented in detail to highlight the likely branchpoint. A polypyrimidine tract, that is typical of sequences that are adjacent to branchpoints, is indicated. The branchpoint consensus sequence, from which the LAT sequence departs, is also shown for comparison.

LAT function

Whatever the mechanism of LAT accumulation, their abundance could be related to a function. One consistent observation is that mutant virus with deletions of the putative major LAT promoter-I and surrounding region reactivate from latent infections less efficiently than the wild type virus (Fraser *et al*, 1992). This implied that LAT are involved in either the establishment of latency or subsequent reactivation. However, the behavior of LAT mutants varies with the model system. For example, the same mutant virus displayed normal reactivation kinetics in a mouse explant reactivation system but reduced reactivation frequency in a rabbit *in vivo* reactivation system (Maggioncalda *et al*, 1994; Hill *et al*, 1996b). Curiously, no specific protein coding information appears to be necessary for LAT function. For example, mutants with disruptions of the open reading frames and other positions within the LAT transcript reactivated from explants of trigeminal ganglia derived from latently infected animals with normal frequency (Block *et al*, 1990; Bloom *et al*, 1994, 1997; Farrell *et al*, 1991). The recognition of open reading frames that could be contained within the large (8.3 kb) LAT transcript suggests that this LAT downstream region is important in virus pathogenesis (Lagunoff *et al*, 1996). Because 2 kb LAT accumulates to such high abundance in the nucleus and is complementary to the 3′-end of the ICP0 transcript, an antisense mechanism of LAT function has been hypothesized (Stevens *et al*, 1987). Recently, in a rabbit *in vivo* system, normal spontaneous reactivation phenotypes could be restored to LAT null mutants by ectopic insertion of the first 1.5 kb of the LAT genomic region (Perng *et al*, 1996a) into the glycoprotein C locus. Since the first 1.5 kb of the LAT region does not specify RNA complementary to ICPO, enthusiasm for an antisense mechanism has been reduced. Therefore, the mechanism of action of LAT remains an enigma.

Mechanism of LAT action and the possible significance of stable, circular RNA

Finally, there are other functions of stable, nuclear RNA that are only now being realized. LAT could serve as a 'sink' for host RNA binding proteins. Also, recent reports indicate that certain abundant RNA species 'invade' DNA chromosomes containing complementary sequences and induce single stranded nicks (Yang *et al*, 1996). This represents an extreme example of how RNA could radically affect a genome (Yang *et al*, 1996). Needless to say, such a behavior by LAT against its gene in the latent HSV chromosome would have an arresting impact upon HSV gene expression, although a mechanism whereby the viral genome repairs itself to enable reactivation must be considered. On the other hand, abundant circular RNA has been reported to induce methylation of cytosine residues (CpG methylation) within regions of DNA homologous to the RNA (Wassenegger *et al*, 1994). Since CpG methylation has been associated with repression of gene transcription, RNA-mediated reductions of methylation of homologous genes could be a means of gene repression (Cedar and Razin, 1990; Cross and Bird, 1995). This model suggests that methylation could be essential to viral reactivation. Using an *in vivo* rabbit model where virus must be 'induced' with catecholamines, Bloom *et al* (1996) and Hill *et al* (1996) have found that HSV mutants with deletions of a genomic region rich in CpG islands, which are predictive for methylation, reactivate *in vivo* with reduced frequency. Dressler *et al* (1987) reported that HSV DNA isolated from latently infected mice is not extensively methylated throughout the genome. However, micro-domains exist in the HSV genome in which methylation can occur. We have recently observed that the LAT region of the HSV-genome, when derived from latently infected tissue is remarkably and specifically resistant to PCR amplification (Su *et al*, 1997). This was not due to the low abundance of HSV DNA in the samples, since the same regions could be efficiently amplified from preparation of lytically infected cells containing the same quantity of viral genome. Therefore, these results suggest that distortions or modifications, such as methylation of the LAT region in the viral DNA from latently, but not productively infected cells, could occur. This hypothesis is being tested (Su *et al*, 1997).

Finally, RNA that accumulates in the nucleus has been shown to direct the methylation of complementary RNA molecules at specific sugar residues (Bailleul, 1996; Cavalle *et al*, 1996; Nicoloso *et al*, 1996). LAT possesses sequences necessary to direct such methylation, and are complementary to other viral transcripts such as ICP0 and these possibilities should be explored.

Conclusions

The accumulation of high concentrations of LAT within the nuclei of latently infected cells could be explained by their (i) stable lariat structure, (ii) continuous gene expression, or (iii) a combination of (i) and (ii). The reason that the LAT lariat is stable could be due to the possession of a poor branchpoint, that serves as a poor substrate for debranching enzymes. Transfection studies (Farrell *et al*, 1991; Wu *et al*, 1996) diminish the possibility that their stability is the result of viral interference with debranching, although this could be an indirect consequence of LAT accumulation. Possibly, LAT accumulation defines a class of neurons with low debranching activity, although the appear-

ance of lariats in productively infected cells makes this less likely (Rodahl and Haarr, 1997; Wu *et al*, 1996; Zabolotny *et al*, 1997).

We speculate that LAT structure and function are related. If LAT do serve to auto-inactivate their own expression (and possibly the expression of other genes), several apparently contradictory facts must be reconciled. First, LAT null mutants have not been reported to 'over-express' viral genes in latently infected cells as would be predicted if LAT function was to be 'repressive' (Bloom *et al*, 1994; Devi-Rao *et al*, 1994). Second, the vast abundance of LAT in latently infected cells would negate the suggestion that their transcription was being repressed in a negative feedback loop. There is evidence that, after a plateau is reached, the LAT in latently infected cells does not increase in abundance over time (Hill *et al*, 1996a) and the LAT promoter is not unusually active during latency (Feldman, 1994). Therefore, LAT within cells could repress their own transcription and accumulate as a consequence of their stability. Thus, proving LAT does not have two ends, there is no end in sight regarding their analysis.

Acknowledgements

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References

Bailleul B (1996). During *in vivo* maturation of eukaryotic nuclear mRNA, splicing yields excised exon circles. *Nucleic Acids Res* **24:** 1015–1019.

Block TM, Deshmane S, Masonis J, Maggioncalda J, Valyi NT, 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.

Block TM, Spivack JG, Steiner I, Deshmane S, McIntosh MT, Lirette RP, Fraser NW (1990). A herpes simplex virus type 1 latency-associated transcript mutant reactivates with normal kinetics from latent infection. *J Virol* **64:** 3417–3426.

Bloom DC (1997). Unpublished results.

Bloom DC, Devi-Rao GB, Hill JM, Stevens JG, Wagner EK (1994). Molecular analysis of herpes simplex virus type 1 during epinephrine induced reactivation of latently infected rabbits *in vivo*. *J Virol* **68:** 1283–1292.

Bloom DC, Hill JM, Wagner EK, Feldman LT, Stevens JG (1996). A 348 base pair region in the latency associated transcripts facilitates herpes simplex virus type 1 reactivation. *J Virol* **70:** 2449–2459.

Bloom DC, Stevens JG, Hill JM, Tran RK (1997). Mutagenesis of a cAMP response element within the latency-associated transcript promoter of HSV-1 reduces adrenergic reactivation. *Virology*, submitted.

Capel BS, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R (1993). Circular transcripts of the testis determining gene Sry in adult mouse testis. *Cell* **73:** 1019–1130.

Cavalle J, Nicoloso M, Bachellerie J (1996). Targeted ribose methylation of RNA *in vivo* directed by tailored antisense RNA guides. *Nature* **383:** 732–735.

Cedar H, Razin A (1990). DNA methylation and development. *Biochem Biophys Acta* **1049:** 1–8.

Cross SH, Bird AP (1995). CpG islands and genes. *Curr Opin Genet Develop* **5:** 309–314.

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 USA* **84:** 3204–3208.

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

Devi-Rao GB, Goodart SA, Hecht LM, Rochford R, Rice MK, Wagner EK (1991). Relationship between polyadenylated and nonpolyadenylated herpes simplex virus type 1 latency-associated transcripts. *J Virol* **65:** 2179–2190.

Dressler GR, Rock DL, Fraser NW (1987). Latent herpes simplex virus type 1 DNA is not extensively methylated *in vivo*. *J Gen Virol* **68:** 1761–1765.

Farrell MJ, Dobson AT, Feldman LT (1991). Herpes simplex virus latency-associated transcript in a stable intron. *Proc Natl Acad Sci USA* **88:** 790–794.

Feldman LT (1994). Transcription of the HSV-1 genome in neurons *in vivo*. *Sem in Virol* **5:** 207–212.

Fraser NW, Block TM, Spivack JG (1992). The latency associated transcripts of herpes simplex virus: RNA in search of function. *Virol* **191:** 1–8.

Hill JM, Garza HH Jr, Su YH, Rapalie M, Hanna LC, Loutsch JM, Thompson HW, Varnell ED, Bloom DC, Block TM (1997). A 437 base pair deletion at the beginning of the LAT promoter significantly reduces adrenergically-induced HSV-1 ocular reactivation in latently infected rabbits. *J Virol*, in press.

Hill JM, Gebhardt BM, Wen R, Bouterie AM, Thompson HW, O'Callaghan RJ, Halford WP, Kaufman HE (1996a). Quantitation of herpes simplex virus type 1 DNA and latency-associated transcripts in rabbit trigeminal ganglia demonstrates a stable reservoir of viral nucleic acids during latency. *J Virol* **70:** 3137–3141.

Hill JM, Maggioncalda JB, Garza HH Jr, Su YH, Fraser NW, Block TM (1996b). *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, Sederati F, Javier RT, Wagner EK, Stevens JG (1990). Herpes simplex virus latent phase transcript facilitates *in vivo* reactivation. *Virology* **174:** 117–125.

Ho DY, Mocarski ES (1989) Herpes simplex virus latent RNA (LAT) is not required for latent infection in the mouse. *Proc Natl Acad Sci USA* **86:** 7596–7600.

Katsafanas GC, Schirmer EC, Wyatt LS, Frankel N (1996). In vitro activation of human herpesviruses 6 and 7 from latency. *Proc Natl Acad Sci USA* **93:** 9788–9792.

Kieff ED (1996). Epstein Barr virus and its replication. In *Fields Virology, Third Edition*. Fields BN, Knipe DM, Howley PM *et al* (eds). Lippincott-Raven Publishers, Philadelphia, pp 2343–2396.

Kosz-Vnenchak M, Jacobson J, Coen DM, Knipe DM (1993). Evidence for a novel regulatory pathway for herpes simplex virus gene expression in trigeminal ganglion neurons. *J Virol* **67:** 5383–5393.

Kramer MF, Coen DM (1995). Quantification of transcripts from the ICP4 and thymidine kinase genes in mouse ganglia latently infected with herpes simplex virus. *J Virol* **69:** 1389–1399.

Lagunoff M, Randall G, Roizman B (1996). Phenotypic properties of herpes simplex virus 1 containing a derepressed open reading frame P gene. *J Virol* **70:** 1810–1817.

Lazinski DW, Taylor JM (1995). Regulation of the hepatitis delta virus ribozyme: to cleave or not to cleave? *RNA* **1:** 225–233.

Maggioncalda J, Mehta A, Fraser NW, Block TM (1994). Analysis of a herpes simplex virus type 1 LAT mutant with a deletion between the putative promoter and the 5′ end of the 2.0-kilobase transcript. *J Virol* **68:** 7816–7824.

Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM (1996). Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology* **225:** 72–81.

Mehta A, Maggioncalda J, Bagasra O, Block TM (1994). A PCR DNA hybridization method to detect HSV-1 DNA neuronal cells *in situ*, derived from latently infected people. *Cell Vision J Morph Analys* **1:** 110–115.

Mehta A, Maggioncalda J, Bagasra O, Thikkavarapu S, Saikumari P, Valyi-Nagy T, Fraser NW, Block TM (1995). *In situ* DNA PCR and RNA hybridization detection of herpes simplex virus sequences in trigeminal ganglia of latently infected mice. *Virology* **206:** 633–640.

Mitchell WJ, Gressens P, Martin JR, DeSanto R (1994). Herpes simplex virus type 1 DNA persistance, progressive disease and transgenic immediate early gene promoter activity in chronic corneal infections in mice. *J Gen Virology* **75:** 1201–1210.

Nicoloso M, Qu LH, Michot B, Bachellerie JP (1996). Intron endoced, antisense small nucleolar RNAs: the characterization of nine novel species points to their direct role as guides for the 2′-O-ribose methylation of rRNAs. *J Mol Biol* **260,** 178–195.

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 (1996a). The spontaneous reactivation function of the herpes simplex virus type 1 LAT gene resides completely within the first 1.5 kbs of the 8.3 kb primary transcript. *J Virol* **70:** 976–984.

Perng G-C, 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.

Qian L, Vu M, Carter M, Wilkinson M (1992). A spliced intron accumulates as a lariat in the nucleus of T cells. *Nucleic Acid Res* **20:** 5340–5345.

Ramakrishnan R, Fink DJ, Jiang G, Desai GP, Glorioso JC, Levine M (1994). Competitive quantitative PCR analysis of herpes simplex virus type 1 DNA and latency associated transcript RNA in latently infected cells of the rat brain. *J Virol* **68:** 1864–1873.

Rodahl E, Haarr L (1997). Analysis of the 2-kilobase latency-associated transcript expressed in PC12 cells productively infected with herpes simplex virus type 1: evidence for a stable, nonlinear structure. *J Virol* **71:** 1703–1707.

Roizman B (1996a). Herpesviridae. In: *Fields Virology, Third Edition*. Fields BN, Knipe DM, Howley PM *et al* (eds). Lippincott-Raven Publishers, Philadelphia, pp 2221–2230.

Roizman B, Sears AE (1996b). Herpes simplex viruses and their replication. In: *Fields Virology, Third Edition*. Fields BN, Knipe DM, Howley PM *et al* (eds). Lipincott-Raven Publishers, Philadelphia, pp 2231–3395.

Steiner IJ, Spivack G, Lirette RP, Brown SM, MacLean AR, Subak-Sharpe J, 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 (1994). Overview of herpes virus latency. *Sem in Virol* **5:** 191–196.

Stevens JG, Cook ML (1971). Latent herpes simplex virus in spinal ganglia of mice. *Science* **173:** 843–845.

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

Straus S (1993). Herpes simplex virus and its relatives. In: Mechanisms of Microbial Disease. Schaecter M, G Medoff, B Eisenstine (eds.) Williams and Wilkins, Publ, Balt, MD.).

Su Y-H, Talsinger R, Fraser NW, Taylor JM, Block TM (1997). Unpublished results.

Trousdale M, Steiner I, Spivack JG Deshmane S, Brown S, Maclean A, Subak-Sharpe J, Fraser NW (1991). Evidence that the herpes simplex virus type 1 latency associated transcripts play a role in reactivation of latent infection *in vivo*. *J Virol* **65,** 6989–6993.

Wagner EK (1994). Herpes simplex virus – moleucular biology. In: *Encyclopedia of Virology*. Webster RG, Granoff A (eds). Academic Press, London, pp 593–603.

Wagner EK, Devi-Rao GB, Feldman LT, Dobson AT, Zhang YF, Flanagan WM, Stevens JG (1988). Physcial characterization of the herpes simplex virus latency-associated transcript in neurons. *J Virol* **62:** 1194–1202.

Wagner EK, Guzowski JF, Singh J (1995). Transcription of the herpes simplex virus genome during productive and latent infection. In: *Progress in Nucleic Acid Research and Molecular Biology (Vol. 51).* Cohn WH, Moldave K (eds). Academic Press, pp 123–165.

Wassenegger M, Heimes S, Riedel L, Sanger HL (1994). RNA directed *de novo* methylation of genomic sequences in plants. *Cell* **76:** 567–576.

Wu TT, Block T, Taylor J (1997). Unpublished results.

Wu T-T, Su Y-H, Block TM, Taylor JM (1996). Evidence that two latency associated transcripts of herpes simplex virus type 1 are non linear. *J Virol* **70:** 5962–5967.

Yang J, Zimmerly S, Perlman PS, Lambowitz AM (1996). Efficient integration of an intron RNA into double stranded DNA by reverse transcriptase. *Nature* **381:** 332–335.

Zabolotny JM, Krummenacher C, Fraser NW (1977). The herpes simplex virus type 1 2.0-kilobase latency associated transcript is a stable intron which branches at a guanosine. *J Virol* **71:** 4199–4208.

Zaphiropoulos PG (1996). Circular RNAs from transcripts of the rat cytochrome P450 2C24 gene: Correlation with exon skipping. *Proc Natl Acad Sci USA* **93:** 6536–6541.