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Short Communication

Tissue specific distribution of the herpes simplex virus type 1 latency-associated transcripts on polyribosomes during latent infection

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Transcription of herpes simplex virus type 1 (HSV-1) during latency produces two abundant latency-associated transcripts (LATs). We have recently shown, that during HSV-1 latency in mice trigeminal ganglia (TG) LATs are bound to polyribosomes (*J Virol*, 1997, 71, 2897–2904). In order to study the possible role of this binding in the latency process, we now extend the polyribosomal analysis to brainstem tissues of latently infected mice, that unlike TG do not support viral reactivation. We report here that the relative amounts of the LATs associated with polyribosomes in the brainstems of mice are significantly lower than those present in TG. We therefore show that binding of the 1.5 and 2.0 kilobases LATs to polyribosomes is tissue specific and hypothesize that this association may have a role in the reactivation function of HSV-1.

Keywords: HSV-1; latency; LATs; polyribosomes

Introduction

Herpes simplex virus type 1 (HSV-1) colonizes the human peripheral sensory ganglia (PSG), establishes latent infection, and can reactivate to produce recurrent mucocutaneous disease (for reviews see Fraser *et al*, 1992; Steiner and Kennedy, 1995). This virus is also responsible for a fatal viral encephalitis, and implicated in the etiology of other neurological conditions of unknown cause such as Alzheimer's (Itzhaki *et al*, 1997) and Behcet's (Eglin *et al*, 1982) disease. Therefore, the molecular phenomena of HSV-1 latent infection in the nervous system have become the focus of intensive research. Much more information is available on the molecular biology of HSV-1 latent infection in the peripheral (PNS) than in the central (CNS) nervous system. During latent infection, viral DNA can be detected in PSG and brain tissues in experimental animals (Rock and Fraser, 1983) as well as humans (Fraser *et al*, 1981; Itzhaki *et al*, 1997; Steiner *et al*, 1988), and it is associated with restricted HSV-1 gene expression (Mitchell *et al*, 1990; Spivack and Fraser, 1987; Stevens *et al*, 1987). The more abundant RNAs detected during HSV-1 latent infection in trigeminal ganglia (TG) are two collinear latency-associated transcripts (LATs), 2.0 and 1.5 kb in size, spanned by RNA of lesser amount, mLAT, the minor hybridizing RNA (Figure 1A and B). Several differences between HSV-1 presence in PNS *versus* CNS tissues have been noted. Only the 2.0 kb (Deatly *et al*, 1990; Steiner *et al*, 1994), but not the 1.5 kb LAT (Steiner *et al*, 1994), is detectable by Northern blot analysis in CNS tissues, and the amounts of the 2.0 kb LAT are much lower than those present during latency in TG. Not less important, explant reactivation from CNS tissues has not been observed, and there is no evidence that under normal conditions HSV-1 can reactivate from CNS tissues in experimental animals or humans (Steiner *et al*, 1994). It is yet unclear whether these transcripts are translated and the mechanism(s) associated with LATs' function are yet unknown.

We have recently demonstrated that the LATs are bound to polyribosomes in TG of latently infected mice (Goldenberg *et al*, 1997). In order to examine whether this observation is relevant to the reactivation function of HSV-1, we compared the associa-

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tion of the two LATs with polyribosomes in nervous tissues that do not facilitate reactivation *versus* tissues from which reactivation is possible, namely brainstem and TG tissues respectively.

Results

Fractionation of polyribosomal RNA from mice TG and brainstem tissues

Typical polysomal profiles after fractionation in 15–45% sucrose gradients are shown in Figure 2. Cytoplasmic extracts from TG of 25 mice (Figure 2A) or from brainstems of six mice (Figure 2B) were fractionated. Fractionation of TG and brainstem polyribosomes was performed in parallel, using tissues from the same infected mice. Polyribosomes from TG tissues could be isolated only after immediate rinsing of the open mouse skull with large volumes of ice-cold buffer. Fractionation of cytoplasmic extract from TG tissue without such cooling, produced profile with only ribosomal subunits and no polyribosomes, similar to that produced by dissociation of polyribosomes with EDTA (Goldenberg *et al*, 1997). This dissociated profile served as a negative control. Five fractions were collected (Figure 2, I to V); in some experiments fractions I to III were used as a single polysomal fraction, and fractions IV and V were combined to produce the subpolysomal fraction, including monosomes, ribosomal subunits and soluble fraction. While the polysomal profile of TG (Figure 2A) contains mostly two to seven ribosomes and only few heavy polysomes, the polysomal profile of brainstem tissue (Figure 2B), which is typical for the brain, consists mainly of heavy polysomes as was noted before (Brown *et al*, 1982). Whether this phenomenon reflects biological differences between the two tissues (different rate of translation or different average length of transcripts), is unclear.

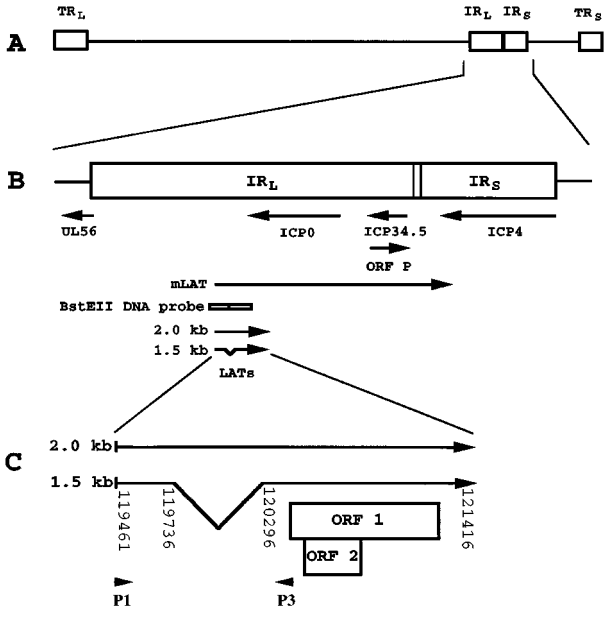

Figure 1 Map of the HSV-1 genome region expressing the LATs and location of primers and probe used. (**A**) Schematic structure of the HSV-1 genome. Inverted repeats (IR - internal, TR - terminal), flanking unique long (TR_L and IR_L) and unique short (TR_S and IR_S) genome regions are indicated. (**B**) The enlarged joint region of HSV-1 genome. Arrows indicate location and orientation of HSV-1 transcripts. Minor latency-associated transcript (mLAT) and two collinear, 2.0 kb and 1.5 kb, latency-associated transcripts (LATs) are the only viral RNAs produced during latency. Two joint open bars indicate the two *BstE*II DNA fragments used as a hybridization probe. (**C**) The 2.0 and 1.5 kb LATs and their two large open reading frames (ORFs). The nucleotide numbers of the 5′ and the 3′ ends of the LATs, and of the splicing boundaries are provided according to the complete HSV-1 DNA sequence in the GenBank (accession number X14112). Location and orientation of the primers P1 and P3 used in this study are indicated by arrows.

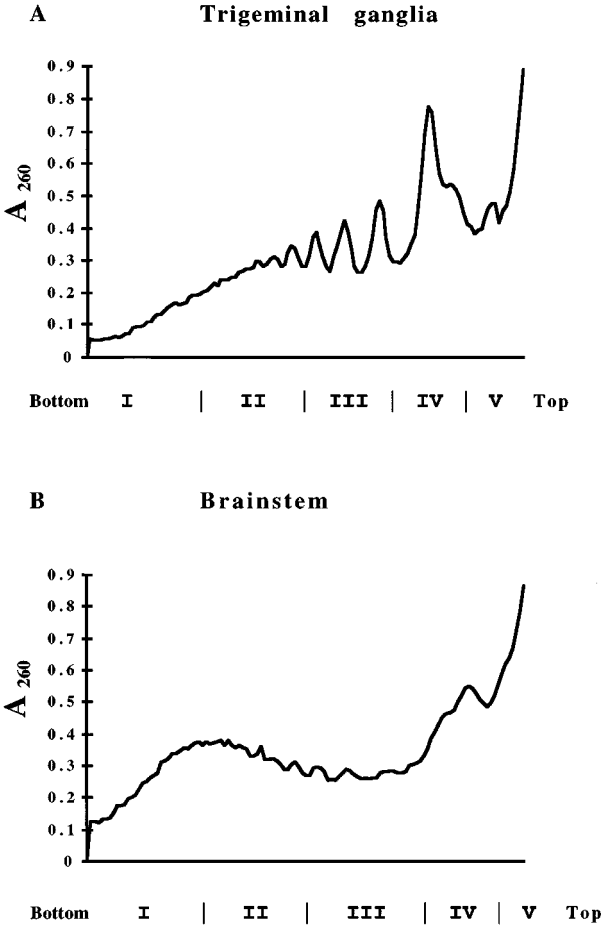

Figure 2 Fractionation and analysis of cytoplasmic RNA from TG and brainstem tissues. Separation analysis by flow spectrophotometry of polyribosomes in the cytoplasmic fractions from (**A**) TG and (**B**) brainstem tissues of mice latently infected with HSV-1 following centrifugation through sucrose gradient (15–45%). Vertical axis – OD_{260}; horizontal axis – fractions, that were usually collected, starting from the bottom of the tube.

Northern blot analysis

The results of Northern blot analysis of polysomal RNA fractions from mice TG and brainstem tissues are shown in Figure 3. Both the 2.0 kb and the 1.5 kb LATs were present in nuclear (Figure 3A, lane 1), polysomal (lanes 2–4) and subpolysomal (lanes 5, 6) RNA fractions from TG of mice latently infected with HSV-1. In contrast, in RNA obtained from the brainstems of the same mice (Figure 3B), the 1.5 kb LAT could not be detected, whereas the 2.0 kb was present in nuclear (lane 1) and subpolysomal (lanes 5, 6), but not in the polysomal (lanes 2–4) RNA fractions.

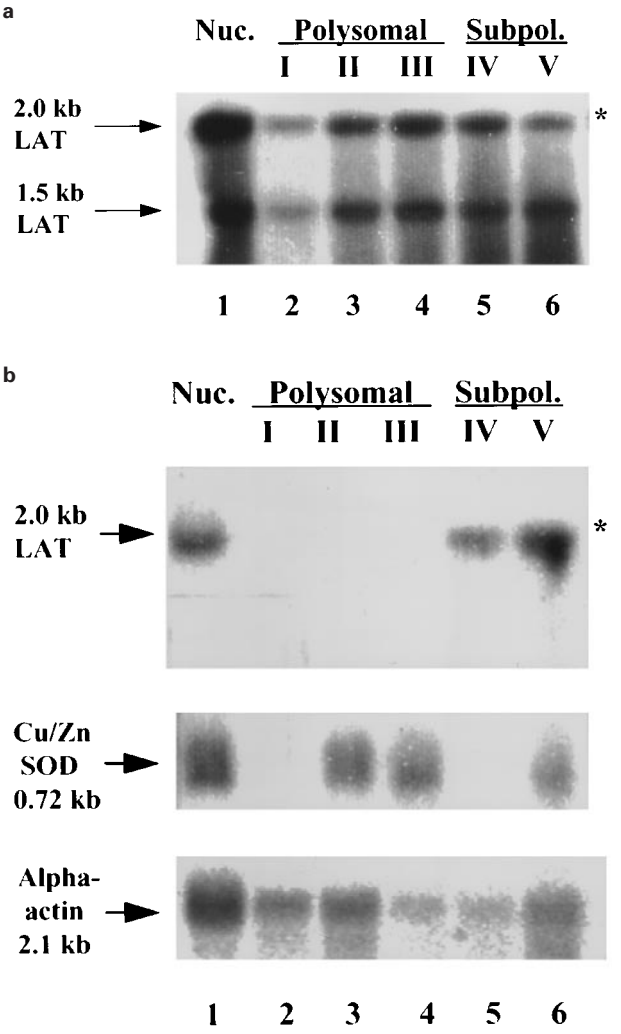

Figure 3 Northern blot analysis of polyribosomal RNA fractions from TG and brainstem tissues of mice latently infected with HSV-1. RNA fractions: lane 1–nuclear RNA; lanes 2, 3, and 4–polysomal RNA (fractions I, II and III in Figure 2, respectively); lanes 5 and 6–subpolysomal RNA (fractions IV and V in Figure 2, respectively). Location of the 18S ribosomal RNA is indicated by an asterisk. (**A**) RNA from TG (5 μg per lane). (**B**) RNA from brainstem tissues (20 μg per lane). Included at the bottom of (**B**) is the same blotted membrane, hybridized with DNA probes specific to Cu/Zn SOD or α-actin.

To validate the separation of polysomal from subpolysomal RNA fractions, we examined the same blots for the distribution of the mRNA of two housekeeping genes, Cu/Zn SOD and α-actin, which are actively translated in all tissues (Benedetto *et al*, 1991; Minty *et al*, 1981). Most of the spliced Cu/Zn SOD 0.72 kb mRNA was located in the middle and light polysomal fractions (Figure 3B, the middle panel, lanes 3 and 4, fractions II and III), as was reported previously (Goldenberg *et al*, 1997), while α-actin mRNA, which is larger than Cu/Zn SOD, was mainly present in the heavy and middle polysomal fractions (Figure 3B, lower panels, lanes 2 and 3, fractions I and II). In RNA from dissociated profile of TG tissue (not subjected to cooling) LATs could be detected only in the subpolysomal, but not in the polysomal fractions (not shown).

Two quantitative features of HSV-1 LATs in mouse brainstem tissue (Steiner *et al*, 1994) are evident in Figure 3B: (1) the 1.5 kb LAT is not detected by Northern blot analysis, and (2) the level of 2.0 kb LAT in brainstem tissue is much lower than in TG (note that while Figure 3A and B represent X-ray films with similar exposure time, each lane in Figure 3A contains only 5 μg of RNA compared to 20 μg in Figure 3B).

The 2.0 kb LAT was readily detectable by Northern blot analysis in the subpolysomal fraction of brainstem tissues obtained from latently infected mice when 10 μg of RNA were loaded (Figure 4A, lane 3). However, in the polysomal fractions, it could be detected by Northern blot analysis of RNAs from brainstems of latently infected mice only when fourfold larger amounts of RNA (40 μg) were loaded on a gel (Figure 4A, lane 7), compared with 10 μg or RNA (lane 2). No LATs were detected in RNA from control uninfected animals (lanes 4–6). The increase in loaded RNA did not enable detection of the 1.5 kb LAT in either the polysomal or subpolysomal RNA (lanes 7–8). Distribution of the Cu/Zn SOD mRNA validated proper separation of polysomal and subpolysomal RNA fractions.

Detection of the 1.5 kb LAT by reverse transcription (RT) PCR and Southern blot analysis

In order to detect specifically the 1.5 kb LAT in brainstem tissues of latently infected mice by a more sensitive approach, we applied RT–PCR analysis. Primers were designed to detect only the spliced 1.5 kb LAT (Goldenberg *et al*, 1997). Southern blot analysis of the RT–PCR products of total, polysomal and subpolysomal RNA from brainstems of mice, using primer pair P1/P3 flanking the intron of the 2.0 kb RNA (Figure 1C), is shown in Figure 4B. The 409 bp DNA fragment, representative of the spliced 1.5 kb LAT, was detected in total, polysomal and subpolysomal fractions of RNA from brainstem tissues of latently infected mice (lanes 3, 5 and 6, respectively). It was absent from similar fractions from uninfected mice (lanes 2, 4, 7 and 8

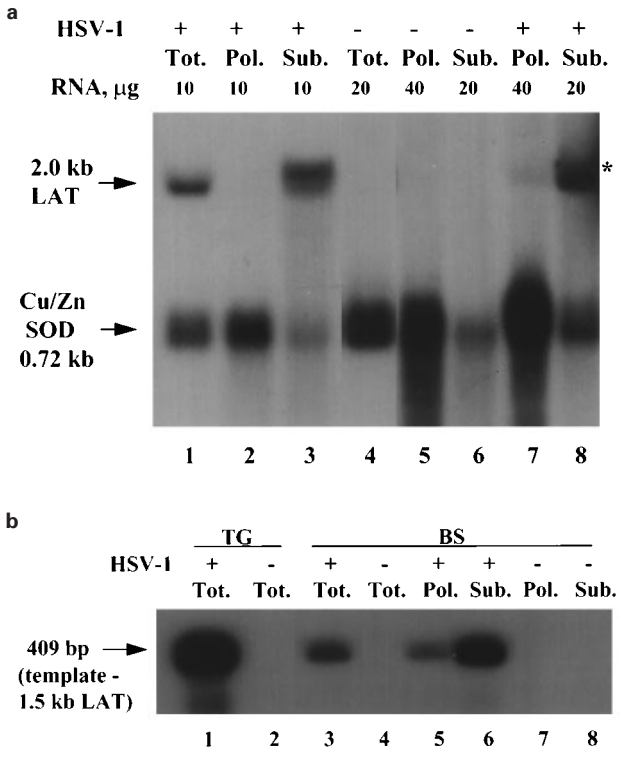

Figure 4 Detection of residual amounts of HSV-1 LATs in polyribosomal RNA fractions from latently infected mice brainstem tissue. (**A**) Northern blot analysis of polyribosomal RNA from brainstem of mice. RNA fractions: Tot.–total RNA, Pol.–combined polysomal RNA (fractions I, II and III in Figure 2), Sub.–combined subpolysomal RNA (fractions IV and V in Figure 2). Lanes 1, 2, 3, 7 and 8–RNA from latently infected mice; lanes 4, 5 and 6–RNA from uninfected mice. Amount of RNA: lanes 1, 2 and 3–10 μg per lane; lanes 4, 6 and 8–20 μg per lane; lanes 5 and 7–40 μg per lane. At the bottom–the same blotted membrane, hybridized with a DNA probe specific to the Cu/Zn SOD gene. (**B**) Southern blot analysis of the RT–PCR products of RNA from TG and brainstem tissues of mice. RT was done using primer P3 and PCR was performed with primers P3 and P1 (see Figure 1C). All designations as in legend to Figure 4A. Lanes 1 and 2–RNA from TG of mice (latently infected with HSV-1 and uninfected, respectively). Lanes 3 to 8–RNA from brainstem of mice (lanes 3, 5 and 6–latently infected with HSV-1 and lanes 4, 7 and 8–uninfected). The 409 bp DNA fragment is representative of the spliced 1.5 kb LAT.

respectively). For each sample, a control without RT enzyme in the reaction failed to identify any band (data not shown). Since the RT–PCR analysis is not quantitative, the intensity of the products shown in Figure 4B do not reflect the actual relative distribution of the 1.5 kb LAT in the total, polysomal and subpolysomal fractions.

Discussion

Our results indicate that both the 2.0 and the 1.5 kb LATs of HSV-1 are present in the cytoplasm of brainstem tissues of latently infected mice, but, in contrast to TG, they are present mainly in the subpolysomal fractions. Only minor amounts of the LATs are bound to polyribosomes of brainstem tissues.

Following the finding that HSV-1 LATs are bound to polyribosomes in mice TG (Goldenberg *et al*, 1997), three questions arise: Does this association have a function in reactivation? Does it result in protein synthesis? What is the mechanism of action of the functional element, be it LATs RNA or its protein product? The present study was aimed to address the first question using the molecular/cellular approach by correlating LATs binding to polysomes with the tissue-specific reactivation phenotype. We chose to examine LATs association to polyribosomes in brainstem tissues since explanted brainstem tissues from both experimental animals latently infected with HSV-1 and humans (which contain viral nucleic acids) do not reactivate virus. We are also unaware of any report of recurrent herpes encephalitis, and this disorder in immunocompetent individuals is a very rare event which occurs at a frequency of 1 million-fold less than the peripheral disease due to recurrent reactivations (Whitley, 1985).

Our results support previous observations (Deatly *et al*, 1990; Steiner *et al*, 1994) that the relative and absolute amounts of HSV-1 LATs in brainstem tissues are much lower than those present in TG. This is even more pronounced with the 1.5 kb LAT, the transcript that is derived from the 2.0 kb LAT by splicing (Spivack *et al*, 1991) and has so far been detected mainly in PSG. The difference in the steady state levels of the 1.5 kb LAT between TG and brainstem tissues may be due to various stability and/or various efficiency of splicing of this RNA in PNS and CNS neurons. Determinants of the 2.0 kb LAT stability–stem-and-loop structure and unusual brand point–are located at the very 3′-end of this transcript (Krummenacher *et al*, 1997), which is common for the 2.0 and 1.5 kb LATs, and these RNAs should therefore have similar stability. Thus, this difference might be due to various activity of splicing factors that produce 1.5 kb LAT from 2.0 kb LAT preferentially in PNS neurons. An association between the production of 1.5 kb LAT and the ability of latent HSV-1 to reactivate from specific tissues has been reported (Tanaka *et al*, 1994), in a study that also demonstrated disappearance of the 1.5 kb LAT during the process of HSV-1 reactivation.

Since the 2.0 kb LAT is a stable intron of mLAT pre-mRNA with an unusual branch point (Zabolotny *et al*, 1997), and LATs are either poorly polyadenylated (Spivack and Fraser, 1987) or not polyadenylated at all (Wagner *et al*, 1988), can they be transported to the cytoplasm? An interesting example supporting such a possibility is the accumulation of intron 1 of the ICP0 gene in the cytoplasm in a stable, nonpolyadenylated form

during HSV-1 infection of cells in culture; this intron RNA appeared to be even more stable than the authentic ICP0 mRNA (Carter and Roizman, 1996).

The present finding that the LATs in brainstem tissues are only barely bound to polyribosomes, and that the levels of the 1.5 kb LAT that are associated with polyribosomes are present at amounts below detection by Northern blot analysis is a further evidence that the LATs may play a role in HSV-1 reactivation ability and that their association with polyribosomes may be important for reactivation.

LATs association with polyribosomes is highly suggestive of translation and synthesis of a functional protein. It was shown, that the splicing to produce the 1.5 kb LAT removes an intron that inhibits translation of the two main LATs' open reading frames (ORFs, Figure 1C) in a transcription-translation system *in vitro* (Spivack *et al*, 1991). Two characteristics of the LATs show that they may code for a protein: (i) the two large LAT's ORFs have several features of typical viral bicistronic mRNAs (discussed in Goldenberg *et al*, 1997) and (ii) the 5′ noncoding region of the LATs is typical for some known tissue-specific mRNAs, whose expression is regulated on the level of translation. There are examples of cell or tissue-specific variations in translational efficiency of eukaryotic mRNAs (Avni *et al*, 1997; Imataka *et al*, 1994; Ranganathan *et al*, 1995; Zimmer *et al*, 1994). In some of them, the transcript has a long 5′-untranslated region containing several AUG codons that precede the major ORF. These upstream AUGs decrease the level of translation of the major ORF in some, but not all, tissues, rendering the translation tissue-specific (Imataka *et al*, 1994; Zimmer *et al*, 1994). This is characteristic for the 2.0 kb LAT (its 960 bp 5′-untranslated region contains nine AUG codons) and, to a lesser degree, for the 1.5 kb LAT (its 400 bp 5′-untranslated region contains two AUG codons), making both LATs good candidates for this mode of tissue-specific regulation of translation. Nevertheless, other possibilities of LATs' function on polyribosomes should also be considered: in some cases translation *per se* might play a role in the turnover of RNAs, as happens with the cellular U22 host gene (Tycowski *et al*, 1996), or with the defective interfering RNAs of the mouse hepatitis virus (van der Most *et al*, 1995).

Further investigation of the function of the association of the LATs with polyribosomes, as well as the localization of the exact region of LAT's sequences that bind to ribosomes, and the search for potential LATs' proteins in a stable neuronal cell line that expresses HSV-1 LATs (Goldenberg *et al*, 1997; Mador *et al*, 1995), is now under way.

Materials and methods

Animals and viruses

Inbred BALB/c female mice (4–5 weeks old) were obtained from Harlan Laboratories (Israel). Mice were infected with HSV-1 strain F (obtained from Dr B Roizman, Chicago) via the cornea as reported (Steiner *et al*, 1989, 1990). Confirmation of latent HSV-1 infection in TG tissues was performed by explant reactivation (Steiner *et al*, 1990). Latently infected mice used in this study were sacrificed 30 or more days after infection.

Preparation of total RNA and isolation of polyribosomal RNA from mice TG and brainstem tissues

Total RNA was isolated from TG and brainstem tissues with Tri-reagent (Molecular Research Center Inc) according to the manufacturer's instructions, treated with RQ1 DNase (Promega), extracted with phenol-chloroform and precipitated by ethanol. Fractionation of polyribosomes and isolation of polyribosomal RNA was done according to the protocol of Meyuhas *et al* (1987), with some modifications as described previously (Goldenberg *et al*, 1997).

Northern blot analysis

Northern blotting of RNA was performed according to Spivack and Fraser (Spivack and Fraser, 1987) with some modifications (Goldenberg *et al*, 1997), using Bio-Rad Model 785 vacuum blotter. RNA markers (281 to 6583 bases) were purchased from Promega Corporation.

Southern blot analysis

DNA fragments were separated on a 2% agarose gel and transferred onto GeneScreen *Plus* membrane (NEN Research Products) as described previously (Goldenberg *et al*, 1997).

RT–PCR analysis

RT was performed using AMV reverse transcriptase (Promega), and PCR was performed with the Taq polymerase of Appligene as described earlier (Goldenberg *et al*, 1997). The DNA primers used for PCR of HSV-1 LATs' region were: **P1** 5′-GGTAGGTTAGACACCTGCTTCTCC (HSV-1 bases 119461-119484); **P3** 5′-GAAAGCATCCTGCCACTGGCATGGA (bases 120428-120404) (see Figure 1C). RT was done with primer P3.

Radioactively labeled DNA probes

As hybridization probes we used the following DNA fragments: (i) a *BstE*II DNA probe (Figure 1B) consisting of two equimolar fragments, 897 bp and 977 bp, that cover the HSV-1 genomic region from nucleotides 119194–121068; (ii) a 198 bp PCR DNA fragment of the housekeeping Cu/Zn superoxide dismutase (SOD) gene (described in Gold-

enberg *et al*, 1997); and (iii) a 1.1 kb *Pst*I DNA fragment of the mouse α-actin gene (Minty *et al*, 1981), obtained from Dr O Meyuhas. All DNA probes were labeled by random priming using the 'multiprime' DNA labeling systems of Amersham as described previously (Goldenberg *et al*, 1997).

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References

Avni D, Biberman Y, Meyuhas O (1997). The 5′ terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. *Nucl Acids Res* **25:** 995–1001.

Benedetto MT, Anzai Y, Gordon JW (1991). Isolation and analysis of the mouse genomic sequence encoding Cu^{2+} – Zn^{2+} superoxide dismutase. *Gene* **99:** 191–195.

Brown IR, Heikkila JJ, Cosgrove JW (1982). Analysis of protein synthesis in the mammalian brain using LSD and hyperthermia as experimental probes. In: *Molecular Approaches to Neurobiology.* Brown IR (ed). Academic Press: New York. pp 221–253.

Carter KL, Roizman B (1996). Alternatively spliced mRNAs predicted to yield frame-shift proteins and stable intron 1 RNAs of the herpes simples virus type 1 regulatory gene α0 accumulate in the cytoplasm of infected cells. *Proc Natl Acad Sci USA* **93:** 12535–12540.

Deatly AM, Haase AT, Fewster PH, Lewis E, Ball MJ (1990). Human herpes virus infections and Alzheimer's disease. *Neuropathol Appl Neurobiol* **16:** 213–223.

Eglin RP, Lehner T, Subak-Sharpe H. (1982). Detection of RNA complementary to herpes simplex virus in mononuclear cells from patients with Behcet's syndrome and recurrent oral ulcers. *Lancet* **2:** 1356–1361.

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

Fraser NW, Lawrence WC, Wroblewska Z, Gilden DH, Doprowski H (1981). Herpes simplex virus type 1 DNA in human brain tissue. *Proc Natl Acad Sci USA* **78:** 6461–6465.

Goldenberg D, Mador N, Ball MJ, Panet A, Steiner I (1997). The abundant latency-associated transcripts of herpes simples virus type 1 are bound to polyribosomes in cultured neuronal cells and during latent infection in mouse trigeminal ganglia. *J Virol* **71:** 2897–2904.

Imataka H, Nakayama K, Yasumoto K, Mizuno A, Fujii-Kuriyama Y, Hayami M (1994). Cell-specific translational control of transcription factor BTEB expression. *J Biol Chem* **269:** 20668–20673.

Itzhaki RF, Lin WR, Shang DH, Wilcock GK, Faragher B, Jamieson GA (1997). Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet* **349:** 241–244.

Krummenacher C, Zabolotny JM, Fraser NW (1997). Selection of a nonconsensus branch point is influenced by an RNA stem-loop structure and is important to confer stability to the herpes simplex virus 2-kilobase latency-associated transcript. *J Virol* **71:** 5849–5860.

Mador N, Panet A, Latchman D, Steiner I (1995). Expression and splicing of the latency-associated transcripts of herpes simplex virus type 1 in neuronal and non-neuronal cell lines. *J Biochem* **117:** 1288–1297.

Meyuhas O, Thompson EA, Perry RP (1987). Glucocorticoids selectively inhibit translation of ribosomal protein mRNAs in P1798 lymphosarcoma cells. *Mol and Cell Biol* **7:** 2691–2699.

Minty AJ, Caravatti M, Robert B, Cohen A, Daubas P, Weydert A, Gros F, Buckingham ME (1981). Mouse actin messenger RNAs. *J Biol Chem* **256:** 1008–1014.

Mitchell WJ, Lirette RP, Fraser NW (1990). Mapping of low abundance latency-associated RNA in the trigeminal ganglia of mice latently infected with herpes simples virus type 1. *J Gen Virol* **71:** 125–132.

Ranganathan G, Ong JM, Yukht A, Saghizadeh M, Simsolo RB, Pauer A, Kern PA (1995). Tissue-specific expression of human lipoprotein lipase. *J Biol Chem* **270:** 7149–7155.

Rock DL, Fraser NW (1983). Detection of HSV-1 genome in the central nervous system of latently infected mice. *Nature* **302:** 523–525.

Spivack JG, Fraser NW (1987). Detection of herpes simplex virus type 1 transcripts during latent infection in mice. *J Virol* **61:** 3841–3847.

Spivack JG, Woods GM, Fraser NW (1991). Identification of a novel latency-specific splice donor signal within the herpes simples virus type 1 2.0-kilobase latency-associated transcript (LAT): translation inhibition of LAT open reading frames by the intron within the 2.0-kilobase LAT. *J Virol* **65:** 6800–6810.

Steiner I, Kennedy PGE. (1995). Herpes simples virus latent infection in the nervous system. *J Neurovirol* **1:** 19–29.

Steiner I, Mador N, Reibstein I, Spivack JG, Fraser NW (1994). Herpes simplex virus type 1 gene expression and reactivation of latent infection in the central nervous system. *Neuropathol and Appl Neurobiol* **20:** 253–260.

Steiner I, Spivack JG, Deshmane SL, Ace CI, Preston CM, Fraser NW (1990). A herpes simplex virus type 1 mutant containing a nontransinducing Vmw65 protein establishes latent infection in vivo in the absence of viral replication and rectivates efficiently from explanted trigeminal ganglia. *J Virol* **64:** 1630–1638.

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.

Steiner I, Spivack JG, O'Boyle II DR, Lavi E, Fraser NW (1988). Latent herpes simplex virus type 1 transcription in human trigeminal ganglia. *J Virol* **62:** 3493–3496.

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

Tanaka S, Minagawa H, Toh Y, Liu Y, Mori R (1994). Analysis by RT–PCR of latency and reactivation of herpes simplex virus in multiple neuronal tissues. *J Gen Virol* **75:** 2691–2698.

Tycowski KT, Shu M-D, Steitz JA (1996). A mammalian gene with introns instead of exons generating stable RNA products. *Nature* **379:** 464–466.

van der Most RG, Luytjes W, Rutjes S, Spaan WJM (1995). Translation but not the encoded sequence is essential for the efficient propagation of the defective interfering RNAs of the coronavirus mouse hepatitis virus. *J Virol* **69:** 3744–3751.

Wagner EK, Flanagan WM, Devi-Rao G, Zhang YF, Hill JM, Anderson KP, Stevens JG (1988). The herpes simplex virus latency-associated transcript is spliced during the latent phase of infection. *J Virol* **62:** 4577–4585.

Whitley RJ (1985). Epidemiology of herpes simplex viruses. In: *The Herpes Viruses,* Roizman B. (ed). Plenum: New York Publishing Corp pp. 1–44.

Zabolotny JM, Krummenacher C, Fraser NW (1997). The herpes simples virus type 1 2.0-kilobase latency-associated transcript is a stable intron which branches at a guanosine. *J Virol* **71:** 4199–4208.

Zimmer A, Zimmer AM, Reynolds K (1994). Tissue specific expression of the retinoic acid receptor-β2: regulation by short open reading frames in the 5′-noncoding region. *J Cell Biol* **127:** 1111–1119.