Available online at www.sciencedirect.com
ScienceDirect
Virology 356 (2006) 106–114
VIROLOGY
www.elsevier.com/locate/yviro

Characterization of a spliced exon product of herpes simplex type-1 latency-associated transcript in productively infected cells

Wen Kang [a], Ruma Mukerjee [a], Jared J. Gartner [a], Artemis G. Hatzigeorgiou [b], Rozanne M. Sandri-Goldin [c], Nigel W. Fraser [a,*]

[a] Department of Microbiology, University of Pennsylvania Medical School, 315 Johnson Pavilion, Philadelphia, PA 19104, USA
[b] Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
[c] Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA 92697-4025, USA

Received 8 May 2006; returned to author for revision 16 June 2006; accepted 21 July 2006
Available online 30 August 2006

Abstract

The latency-associated transcripts (LATs) of herpes simplex virus type-1 (HSV-1) are the only viral RNAs accumulating during latent infections in the sensory ganglia of the peripheral nervous system. The major form of LAT that accumulates in latently infected neurons is a 2 kb intron, spliced from a much less abundant 8.3 primary transcript. The spliced exon mRNA has been hard to detect. However, in this study, we have examined the spliced exon RNA in productively infected cells using ribonuclease protection (RPA), and quantitative RT-PCR (q-PCR) assays. We were able to detect the LAT exon RNA in productively infected SY5Y cells (a human neuronal cell line). The level of the LAT exon RNA was found to be approximately 5% that of the 2 kb intron RNA and thus is likely to be relatively unstable. Quantitative RT-PCR (q-PCR) assays were used to examine the LAT exon RNA and its properties. They confirmed that the LAT exon mRNA is present at a very low level in productively infected cells, compared to the levels of other viral transcripts. Furthermore, experiments showed that the LAT exon mRNA is expressed as a true late gene, and appears to be polyadenylated. In SY5Y cells, in contrast to most late viral transcripts, the LAT exon RNA was found to be mainly nuclear localized during the late stage of a productive infection. Interestingly, more LAT exon RNA was found in the cytoplasm in differentiated compared to undifferentiated SY5Y cells, suggesting the nucleocytoplasmic distribution of the LAT exon RNA and its related function may be influenced by the differentiation state of cells.

Keywords: HSV-1; LAT; Exon

Introduction

Herpes simplex virus type-1 is a neurotropic virus and establishes life long latent infections in the sensory ganglia of the peripheral nervous system (for review see (Wagner and Bloom, 1997). During latency, the latency-associated transcripts (LATs) are the only abundantly transcribed RNA (for review see Fraser et al., 1992). The LAT region has been implicated to play a role in promoting efficient establishment of latency and viral reactivation (for review see Kent et al., 2003). The major form of LAT is a 2 kb intron, which is spliced from an 8.3 primary transcript (Farrell et al., 1991). However, the spliced 6.3 kb exon RNA has been hard to detect by any method. Previous studies using Northern blot analysis and ribonuclease protection assay failed to detect the spliced LAT exon RNA (Devi-Rao et al., 1991; Dobson et al., 1989). It has been speculated that that the LAT exon is either expressed at a very low level or undergoes rapid degradation in tissue. Using sensitive RT-PCR assays, a number of groups have been able to detect the spliced LAT exon in productively infected cells (Alvira et al., 1999; Krummenacher et al., 1997). However, a complete characterization of the LAT exon RNA remains to be performed.

Several lines of evidence recently indicate that the LAT exon region may play a role in the life cycle of HSV-1. The 5′1.5 kb region of LAT has been implicated in promoting cell survival through an anti-apoptotic mechanism (Ahmed et al., 2002; Jin et al., 2003; Perng et al., 2000). This region is also known to be

* Corresponding author. Fax: +1 215 898 3849.
E-mail address: nfraser@mail.med.upenn.edu (N.W. Fraser).

0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.07.033

involved in supporting long-term transgene expression (Lokensgard et al., 1997). In addition to its enhancer activity, the LAT exon 1 has also been reported to function as a region responsible for keeping the LAT promoter active during latency (Berthomme et al., 2001).

It is unknown whether protein translation from the LAT exon mRNA occurs during infection. Although it has been suggested that large protein products translated from the first 1.5 kb LAT are unlikely (Drolet et al., 1998), polypeptides such as ORF P may be translated from the LAT exon II region under certain circumstances (Bruni and Roizman, 1996). In addition, previous work has shown that a transgene inserted in the exon 1 region of the LAT was translated in some CNS neurons in mice during latency (Zhu et al., 2000), suggesting that protein translation in the exon 1 region is possible, especially in certain types of neurons during latency. Alternatively, the LAT exon transcript may function as a regulatory RNA (Inman et al., 2001), since it is transcribed from the opposite strand of viral immediate early gene ICP0, and it has been speculated that it may down-regulate the expression of ICP0 by an antisense mechanism (Stevens et al., 1987). In fact, LAT has been shown to inhibit the transactivating activity of ICP0 in transient transfection assays (Farrell et al., 1991). Furthermore, several reports suggest that LAT suppresses viral replication and reduces viral mRNA levels (Chen et al., 1997; Mador et al., 1998). Recent studies performed in our lab indicate that the exon 1 of the LAT gene encodes an miRNA that plays an active role in regulating apoptosis in the infected cell (Gupta et al., 2006).

In the present study, we have examined the expression of the LAT exon mRNA in productively infected cells. Using a ribonuclease protection assay, we quantified the LAT exon RNA in productively infected cells. It was found that the LAT spliced exon RNA was present at a level that is approximately 5% of that of the 2 kb LAT intron on a molar basis. The LAT exon mRNA appeared to be mainly retained in the nucleus of productively infected cells during the late stage of an infection. Interestingly, the LAT mRNA appeared to favor a more cytoplasm distribution in differentiated compared to undifferentiated SY5Y cells, indicating that the distribution of the LAT exon RNA and its related function may be influenced by the differentiation state of the infected cell.

Results

Detection of LAT exon RNA by RPA

In an attempt to detect LAT exon RNA, Northern blot analysis was first performed. However, we were unable to detect any specific signals associated with the LAT exon RNA (data not shown). Thus we employed an alternative approach, and examined the presence of the LAT exon RNA by a more sensitive method—ribonuclease protection assay (RPA). To allow the detection and quantitation of the LAT exon RNA, a plasmid (Lex2) encoding a 245 bp fragment of the LAT exon I–exon II junction was constructed as described in Materials and methods and used for in vitro transcription of RNA antisense to the LAT exon. To detect the 2 kb LAT intron RNA, a fragment consisting a 265 bp HSV-1 DNA (HSV-1 nt 119382 to nt 119647) was cloned to generate the plasmid Dex1 and used for in vitro transcription of RNA antisense to the LAT intron. Control plasmids were also made in which the inserts were cloned in the opposite orientation, and sense RNAs to the LAT were made following *in vitro* transcription under the T7 promoter.

SY5Y cells were infected with HSV-1 strain F and total RNA was isolated 16 h post-infection. RPA assays were performed on the total RNA with the LAT exon and intron probes. The results are shown in Fig. 1. A protected fragment of 245 bases, corresponding to the spliced LAT exon, was seen with RNA from infected SY5Y cells, but not in mock infected SY5Y cells or with yeast control RNA (Fig. 1A). The 182 nt band representing the LAT intron was readily detected in infected SY5Y cell RNA (Fig. 1B). Both LAT exon and intron signals intensified with increasing amounts of RNA from infected cells. However, the intensity of the LAT exon band appeared to be much weaker than that of the LAT intron when equal amounts of probes were included in all hybridization reactions. A quantification on the intensity of bands after being adjusted for the probe length suggested that the level of the LAT exon was approximately 5% of that of the LAT intron, and therefore,

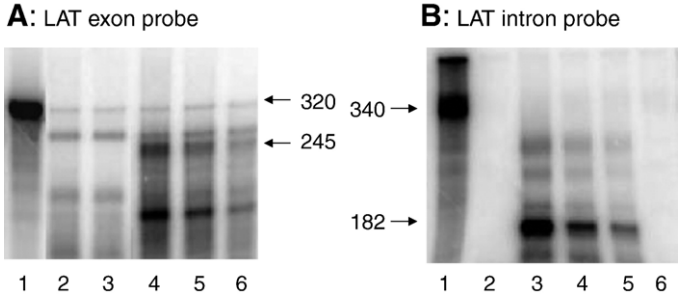

Fig. 1. Detection of LAT exon and intron RNA in productively infected SY5Y cells by RPA. SY5Y cells were infected with HSV-1 strain F (moi = 3) and total RNA was extracted 16 h post-infection. ^{32}P-labeled riboprobes for RPA were generated by *in vitro* translation of linear plasmid templates under the control of the T7 promoter. Protected fragments were resolved by 6% Urea-TBE polyacrylamide gel electrophoresis and gel images analyzed by a phosphoimager. Lane labels in panel A are: (1) LAT exon probe; (2) 10 μg yeast RNA; (3) 20 μg mock SY5Y RNA; (4) 40 μg infected SY5Y RNA; (5) 20 μg infected SY5Y RNA; (6) 10 μg infected SY5Y RNA. Lane labels in panel B are: (1) LAT intron probe; (2) 10 μg yeast RNA; (3) 40 μg infected SY5Y RNA; (4) 20 μg infected SY5Y RNA; (5) 10 μg infected SY5Y RNA; (6) 20 μg mock SY5Y RNA.