A herpes simplex virus type 1 variant, deleted in the promoter region of the latency-associated transcripts, does not produce any detectable minor RNA species during latency in the mouse trigeminal ganglion

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In peripheral sensory ganglia latently infected with herpes simplex virus type 1 (HSV-1) transcription is restricted. A set of viral latency-associated transcripts, the LATs, have been characterized by Northern blotting and in situ hybridization. These transcripts have previously been mapped to a 3 kb region of the viral genome within the repeat long region. However, transcription from adjacent regions of the genome can be detected by in situ hybridization, which cannot be detected by Northern blotting. These RNAs are termed minor LATs or m-LAT. In this study we show that in ganglia latently infected with the HSV-1 variant 1704, which is deleted in one complete copy of the LAT gene and in the promoter and 5' portion of the other copy, m-LATs are not detected by in situ hybridization. Furthermore, the levels of DNA in nervous system tissue latently infected with the parental and the 1704 variant virus are similar. Thus we propose that the sequence elements necessary for initiating transcription or stabilizing m-LATs are within the region deleted in variant 1704 that codes for the promoter and the 5' end of the LATs.
shown to establish latency and to reactivate (Steiner et al., 1989). Similarly, other studies have reported that mutant viruses which do not produce detectable levels of LAT establish latency and reactivate (Javier et al., 1988; Leib et al., 1989). The m-LAT was not specifically studied in these reports. The only probe used in these reports that contained sequences coding for m-LAT, without accompanying LAT sequences, was negative for ganglia latently infected with wild-type virus. Therefore, it is uncertain whether the probes that contained both LAT and m-LAT sequences would have detected m-LAT. In the present study we have examined HSV-1 variant 1704 in latently infected mice trigeminal ganglia to determine whether the deletions in the LATs would affect the production of the m-LATs.

Female BALB/c mice (4 to 6 weeks old) were inoculated in each eye with 1 x 10^6 p.f.u. of either HSV-1 1704 or HSV-1 17+ after corneal scarification. Virus stocks were prepared as described previously (Deatly et al., 1988; Spivack & Fraser, 1987). Latently infected mice (4 weeks post-infection) were killed and trigeminal ganglia were removed for explant cocultivation, DNA extraction and in situ hybridization. From one group of mice ganglia were cocultivated with CV-1 cells to monitor reactivation. Reactivation was determined by the presence of c.p.e. in the CV-1 cell monolayer. Ganglia were transferred to fresh CV-1 cells after 5 to 7 days and observed for reactivation over a 35 day period. From another group of ganglia (six/sample) DNA was extracted as described previously (Mellerick & Fraser, 1986) and spotted in 5 gg samples onto nitrocellulose in the presence of 6 x SSC. DNA from brainstems of latently infected mice was prepared as described for the ganglia. The nitrocellulose was baked for 2 h at 80 °C and the filters were prehybridized. Hybridization was with 32P-labelled (nick-translated) HSV-1 F genome DNA. After washing, the filters were autoradiographed with XAR-5 film and intensifying screens at -70 °C.

For in situ hybridization ganglia were fixed in paraformaldehyde–lysine–periodate (Deatly et al., 1988) for 24 h and then stored in 70% ethanol. The paraffin-embedded ganglia were sectioned at 6 gin, affixed to slides, deparaffinized and treated with Proteinase K. The sections were hybridized with nick-translated (Maniatis et al., 1982) 35S-labelled DNA probes [HSV-1 genomic DNA, KpnI–BamHI, BamHI–SacI and SacI–SacI (4-2 kb)] at 50 °C in hybridization buffer as described previously (Deatly et al., 1987, 1988). DNA for probes was derived from purified HSV-1 (F) virion DNA or purified as restriction fragments (Deatly et al., 1988).
from recombinant plasmids containing the *BamHI* B or *BamHI* SP fragments of HSV-1 (F) (Post et al., 1980). Tissues were washed as described (Deatly et al., 1987, 1988), except that the first wash was at 50 °C for 4 h. Hybridized sections were autoradiographed in NTB-2 emulsion (Kodak) for 4 to 10 days, developed (D19 Kodak) and stained with haematoxylin and eosin. Four mice (two ganglia per mouse) latently infected with either variant 1704 or parental strain 17+ and at least eight sections per ganglion were examined for hybridization with each of the probes described above. Ganglia sections from uninfected mice and HSV-1 strain F latently infected mice were used as negative and positive controls in each experiment.

Reactivation was detectable at explant in all four mice latently infected with strain 17+ (seven of eight ganglia) and in six of seven mice (12/14 ganglia) latently infected with 1704. The reactivation of latent 1704 was significantly delayed when compared to strain 17+, confirming our previous study (Steiner et al., 1989). Ganglia latently infected with strain 17+ reactivated 7 to 9 days post-explantation, but with 1704 reactivation was first detected on day 9 and was present in 50% of ganglia at day 14. Twelve of 14 ganglia latently infected with 1704 yielded infectious virus by day 22 post-explantation.

In order to determine whether the slow rate of reactivation of 1704 was due to reduced amounts of latent viral genomes we examined the levels of viral DNA in latently infected tissue. By spot blot analysis similar amounts of viral DNA were detected in ganglia of mice latently infected with strain 17+ and 1704 (Fig. 2). Viral DNA detected in latently infected 1704 ganglia was $10^{-4}$ to $10^{-5}$ μg per six ganglia and viral DNA detected in latent infected 17+ ganglia was $10^{-5}$ to $10^{-6}$ μg per six ganglia. Viral DNA in brainstems of mice latently infected with 1704 virus was also similar in amount to 17+. Thus variant 1704 behaved like 17+ virus with respect to the amount of viral DNA maintained in latently infected peripheral and central nervous system tissue. These results are in agreement with the DNA levels found for other LAT− viruses (Leib et al., 1989; Sedarati et al., 1989).

We could not detect any m-LAT by *in situ* hybridization in 1704 latently infected ganglia with $^{35}$S-labelled DNA fragments corresponding to the domain of the minor hybridizing RNA [KpnI–BamHI, BamHI–SacI and SacI–SacI (4-2 kb)] (Fig. 3a to e). To test the possibility that other regions of the viral genome were expressed a total HSV-1 genome DNA probe was hybridized to tissue from trigeminal ganglia latently infected with 1704 (Fig. 3d). No hybridization signal was present with exposures as long as 10 days (data not shown). In the same experiments with all the DNA probes described above HSV-1 strain 17+ (Fig. 3e to h) and strain F latently infected ganglia were positive for viral RNA in ganglionic neurons of all mice examined, whereas uninfected ganglia were always negative.

Based upon the data from these experiments it seems very likely that the deletions in the HSV-1 variant 1704 have resulted in disruption of regulatory or structural elements that are necessary for the production of detectable levels of m-LAT. These data, together with other experiments showing that the *KpnI–BamHI* and *BamHI–SacI* portion of m-LAT is transcribed in a rightward direction (Mitchell et al., 1990), seem to indicate that the m-LAT initiates from a point near or coinciding with the start site for the LAT, or that the regulatory elements for the respective RNAs are within the 1704 deletion. An alternative but less likely possibility is that the deletion in 1704 disrupts some function, which secondarily affects the production (e.g. transactivation) or stability of the minor hybridizing RNA (m-LAT).

Since the amounts of viral DNA in 1704 and 17+ latently infected ganglia are similar (Fig. 2) it is probable...
Fig. 3. In situ hybridizations of HSV-1-infected mice trigeminal ganglia. Panels (a) to (d) are ganglia from mice latently infected with strain 1704 (4 weeks post-infection) and panels (e) to (h) are ganglia from mice latently infected with strain 17÷ (4 weeks post-infection). The probes (see Fig. 1) were KpnI-BamHI (a and e), BamHI-SacI (b and f), 4.2 kb SacI-SacI (c and g) and HSV-1 genome (d and h). Exposures were for 4 days. Additional slides of 1704 with each probe were exposed for 10 days with no detection of a signal. Bar marker represents 100 µm for (a) to (g) and 50 µm for (h).

that the decreased or absent m-LAT is not the result of fewer latently infected cells or of latently infected cells containing fewer copies of viral DNA. This finding also supports the possibility that the delayed reactivation kinetics (Steiner et al., 1989) observed with 1704 may be the result of some biological function affected by the deletions, rather than a difference in the number of genome copies present during latency. The biological difference could involve decreased or absent levels of m-LAT, or LAT, or of some other interacting function between putative products of the two. It is also possible that unknown viral products other than LAT and m-LAT, or their possible products, are affected by the deletion and may lead to the observed delay in reactivation. Finally, based upon the findings of no detectable viral RNA in strain 1704 latently infected ganglia, and the ability of 1704 to establish a latent infection that can be reactivated, it seems likely that viral RNA transcription during latency, at a level detectable by in situ hybridization, is not necessary for establishment or reactivation of latent infections. These findings suggest that transcripts associated with latency, the LAT which have previously been shown not to be essential for latency (Javier et al., 1988; Steiner et al., 1989; Leib et al., 1989) and the m-LAT described in this paper are not necessary for the establishment or reactivation of latent infections. More importantly, the data indicate that the sequence elements necessary for transcription of m-LAT are located within the 1-2 kb deletion in BamHIE between nucleotides 7202 and 8144 (Steiner et al., 1989) in HSV-1 variant 1704.

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References


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