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A Viral Function Represses Accumulation of Transcripts from Productive-Cycle Genes in Mouse Ganglia Latently Infected with Herpes Simplex Virus

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Latent infections of neurons by herpes simplex virus form reservoirs of recurrent viral infections that resist cure. In latently infected neurons, viral gene expression is severely repressed; only the latency-associated transcripts (LATs) are expressed abundantly. Using sensitive reverse transcriptase PCR assays, we analyzed the effects of a deletion mutation in the LAT locus on viral gene expression in latently infected mouse trigeminal ganglia. The deletion mutation, which reduced expression of the major LATs 10^5-fold, resulted in a ~5-fold increase in accumulation of transcripts from the immediate-early gene encoding ICP4, an essential transactivator of viral gene expression. The LAT deletion also resulted in a >10-fold increase in the accumulation of transcripts from the early gene encoding thymidine kinase, whose expression during productive infection stringently depends on ICP4, and positively affected the correlation of the levels of these transcripts with the levels of *ICP4* transcripts. We also detected transcripts antisense to *ICP4* RNA, which were in substantial excess to *ICP4* transcripts in ganglia latently infected with wild-type virus. In contrast to its effects on productive-cycle transcripts, the LAT deletion reduced the accumulation of these antisense transcripts ~15-fold. Thus, a viral function associated with the LAT locus represses the accumulation of transcripts from at least two productive-cycle genes in latently infected mouse ganglia. We discuss possible mechanisms and consequences of this repression.

The interaction of herpes simplex virus (HSV) with the mammalian nervous system that results in virus latency is probably the most fascinating biological property of the virus. Because latent virus evades immune surveillance yet reactivates to cause recurrent disease, latency is the most distinctive medical feature of herpesvirus infection. How does a virus that expresses and replicates its genome so efficiently during productive infection become so dormant during latency? During productive infection, immediate-early (IE), early (E), and late (L) viral genes are abundantly expressed in an ordered cascade that culminates in the replication and packaging of viral DNA into infectious particles. In particular, the IE protein, ICP4, is crucial for efficient expression of E and L genes (21, 49, 65). However, during latent infection of sensory neurons, the only abundant gene products detected are the latency-associated transcripts (LATs) (62). Several LAT species have been identified (7, 35, 43, 58–60, 62, 64, 69). They derive from the DNA strand opposite that which encodes ICP0 (Fig. 1), an IE regulatory protein that is important but not essential for viral replication or for reactivation from latency (8, 13, 27, 37, 53, 63). Upon their discovery, it was hypothesized that the LATs function to maintain latency (62). However, subsequent studies have yet to provide direct evidence to support this hypothesis or to identify a specific molecular effect of the LATs in ganglia latently infected with HSV. Indeed, the LATs have been described as "RNA in search of function" (25), and consideration has been given to the hypothesis that latency is controlled solely by the neuron rather than the virus (30, 52, 56).

Previously, we developed sensitive reverse transcriptase PCR (RT-PCR) assays to quantify viral nucleic acids in latently infected trigeminal ganglia (33). Aside from high-level expression of LATs, we found transcripts specific to the *ICP4* gene and to the E gene encoding thymidine kinase (TK) in most ganglia. In a small number of ganglia latently infected with a LAT deletion mutant, we obtained preliminary evidence of higher levels of *ICP4* and *tk* RNA than those detected in ganglia latently infected with wild-type (wt) HSV-1 strain KOS (33). In this study, we investigated this phenomenon further and found that a viral function associated with the LAT locus acts to decrease expression of *ICP4* and *tk* transcripts in latently infected mouse ganglia while increasing expression of RNA antisense to mRNA encoding ICP4.

MATERIALS AND METHODS

Viruses and cells. Wild-type HSV-1 strain KOS and LAT deletion mutant *dl*LAT1.8 and its rescued derivative FSLAT$^+$ (36) were propagated and assayed on Vero cell monolayers as described previously (14). The location of the deletion mutation in *dl*LAT1.8 is shown in Fig. 1.

Infection of mice and tissue collection. Animal experiments were conducted in accordance with institutional guidelines at the Dana-Farber Cancer Institute. Eight-week-old male CD-1 mice (Charles River Laboratories) were anesthetized and then inoculated with 2×10^6 PFU of virus or mock infected with virus diluent via corneal scarification (37). At 30 days postinoculation, trigeminal ganglia were removed and rapidly frozen in liquid nitrogen as previously described (33).

RT-PCR analysis. Ganglionic DNA and RNA were prepared and analyzed by PCR and RT-PCR by using the primers, probes, and conditions described previously (33) except that in some cases amounts of β-actin RT-PCR products were

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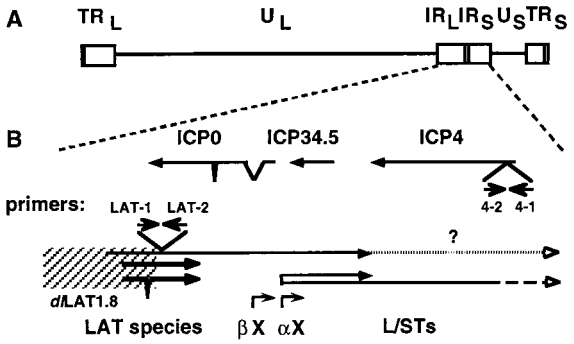

FIG. 1. Locations of relevant HSV transcription units. (A) Diagram of the prototype HSV genome, with the long and short unique (U_L and U_S) sequences (lines) bracketed by the terminal and internal repeat (TR and IR) regions (boxes). (B) Expanded diagram of the IR region showing relative sizes, locations, and orientations of transcripts encoded in this region, with bent lines indicating sequences removed by splicing. The locations and orientations of the LAT-1, LAT-2, 4-1, and 4-2 primers used in this study are shown. Major and minor LAT species are indicated with thick and thin lines, respectively, and the locations of only the two most abundant L/ST species are shown (66). The dotted line with the question mark above it indicates a possible provenance for Anti-4 transcripts during latency. The dashed line on the longer L/ST transcript and the open arrows at the 3′ end of this transcript and the potential Anti-4 transcript indicate that the precise 3′ ends of these transcripts are not established. The stippled box encompassing the 5′ end of LAT and extending upstream represents the location of the ~1.8-kb deletion in *dl*LAT1.8, which is restored in FSLAT$^+$ (36). This deletion removes the promoter, transcriptional start site, and 1,015 bp of transcribed sequences of LAT. The locations of the βX and αX promoters (4) are shown as bent arrows.

quantified by analysis of gels stained with SYBR Green 1 (Molecular Probes, Eugene, Oreg.), using a FluorImager (Molecular Dynamics, Sunnyvale, Calif.). The locations of primers LAT-1, LAT-2, 4-1, and 4-2 are shown in Fig. 1. For analysis of transcripts antisense to *ICP4* mRNA (Anti-4), cDNA was prepared from ganglionic RNA by using primer 4-1, PCR was performed with primers 4-1 and 4-2 as described previously (33), and the signals following hybridization with primer 4-3 (33) were compared with that obtained by using RNA transcribed from plasmid pKS+5′ICP4 (33) by T7 RNA polymerase as described previously (33). To demonstrate our ability to quantify *ICP4* and Anti-4 transcripts from the same sample, we combined synthetic *ICP4* and Anti-4 RNAs in various molar ratios and in absolute amounts comparable to what is measured in infected tissue and mixed these synthetic RNAs with mouse brain RNA. Separate portions of the mixtures were reverse transcribed by using either the ICP4 5′ primer, 4-1, to produce Anti-4 cDNA or the 3′ primer, 4-2 (Fig. 1B), to generate *ICP4* cDNA. The cDNAs were then amplified by PCR using the 4-1–4-2 primer pair and quantified as described previously (33). A linear standard curve was obtained when synthetic Anti-4 RNA was varied by serial 10-fold dilutions, whether alone (Fig. 2A) or in the presence of a constant level of *ICP4* RNA (Fig. 2B, top panel). As shown in the bottom panel of Fig. 2B and plotted in Fig. 2C, quantification of *ICP4* RNA remained unaffected even when the amount of synthetic Anti-4 RNA was varied over 6 orders of magnitude. Experiments to establish the strand specificity of the assays showed that assays using primer 4-2 (Fig. 1) and synthetic Anti-4 failed to generate a signal unless $\geq 10^6$ molecules of Anti-4 were present (11). This signal, at approximately 0.01% of that found with primer 4-1, is probably an artifact of in vitro transcription resulting from the generation of complementary transcripts by bacteriophage RNA polymerases (31, 40, 54) and did not affect the quantification of ganglionic transcripts. Similar studies confirmed that quantification of Anti-4 RNA was unaffected by various amounts of *ICP4* RNA and established the strand specificity of the *ICP4* assay (11). Thus, neither *ICP4* RNA nor Anti-4 RNA contributes to or interferes with the other's signal in our assays.

RESULTS

Effects of a LAT deletion on levels of viral DNA and LATs in mouse ganglia. To investigate the effects of a LAT deletion on the accumulation of viral DNA and RNAs in mouse ganglia latently infected with HSV, we inoculated mice via the corneal route with equal numbers of PFU of either (i) wt virus, (ii) a LAT deletion mutant, *dl*LAT1.8, in which the LAT promoter and 5′ transcribed sequences were removed (Fig. 1), or (iii) a rescued virus, FSLAT$^+$, in which the sequences deleted in *dl*LAT1.8 were restored (36). At 30 days postinoculation (when latency had been established), individual trigeminal ganglia were assayed for HSV DNA by PCR and for LATs and other virus-specific transcripts by RT-PCR. The viral DNA in individual ganglia was normalized to the amount of mouse cellular DNA by assaying for the single-copy gene encoding adipsin (30), and virus-specific transcripts were normalized to mouse cellular RNA by assaying for mRNA encoding β-actin

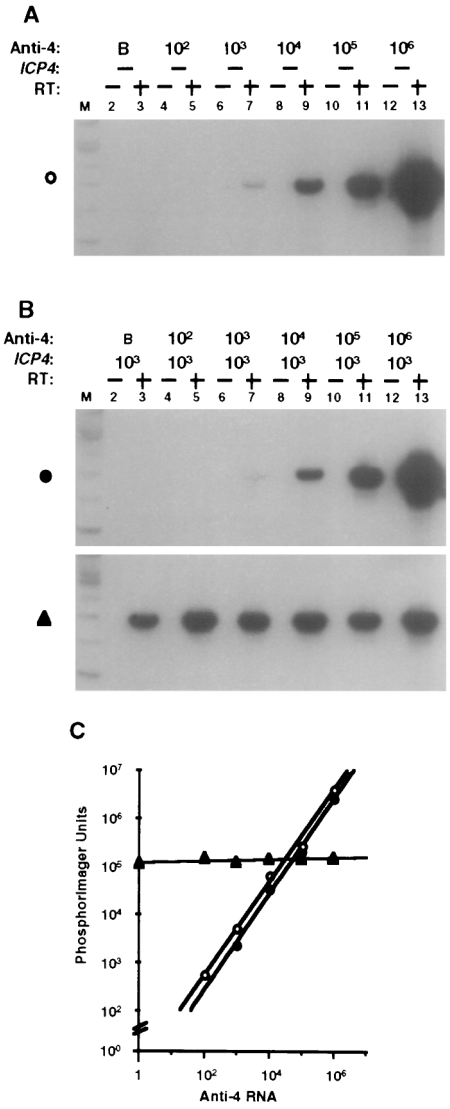

FIG. 2. Validation of Anti-4 RT-PCR assay. (A) Southern blot of a representative Anti-4 standard curve. Serial dilutions of synthetic Anti-4 RNA were combined with uninfected mouse brain RNA. Half of each sample was incubated without (−) or with (+) reverse transcriptase (RT), using the ICP4 5′ primer 4-1, amplified by PCR, and then run in adjacent lanes. Gels were transferred, probed, and quantified as described previously (33). M, marker; B, uninfected mouse brain RNA alone. (B) Southern blots of Anti-4 and *ICP4* synthetic RNAs assayed from mixtures of the two. Serial dilutions of synthetic Anti-4 RNA were mixed with a constant amount of synthetic *ICP4* RNA. Portions of each mixture were hybridized with either primer 4-1 (top) or 4-2 (bottom); half of each sample was reversed transcribed without or with reverse transcriptase to produce cDNAs and then amplified by PCR (33). (C) The Southern blots from panels A and B were quantified with a PhosphorImager. Log_{10} values of PhosphorImager units were plotted against the log_{10} number of Anti-4 RNA molecules (A; open circles; B, top panel; filled circles) or *ICP4* RNA molecules (B, bottom panel; triangles). Best-fit lines were generated by linear regression analysis.

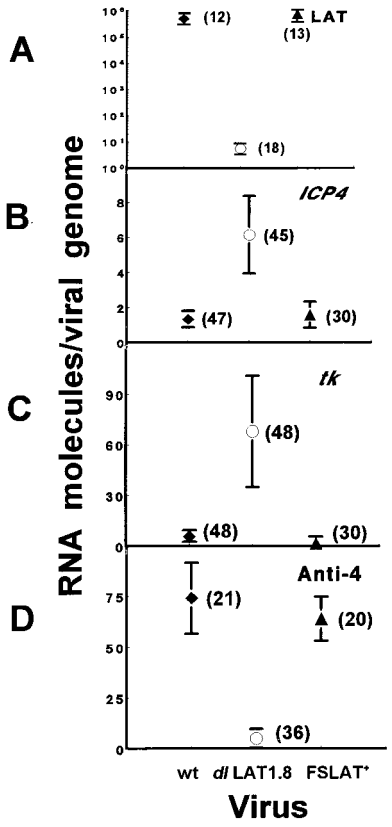

FIG. 3. Amounts of HSV-specific RNAs in mouse ganglia latently infected with HSV-1 strain KOS (diamonds), *dl*LAT1.8 (circles), or FSLAT$^+$ (triangles). (A) Average number of LAT transcripts per viral genome; (B) average number of *ICP4* transcripts per viral genome; (C) average number of *tk* transcripts per viral genome; (D) average number of Anti-4 transcripts per viral genome. Values are expressed as number of RNA molecules normalized to cellular mRNA encoding β-actin, per number of viral genomes normalized to cellular DNA, and represent the results of four independent experiments. The total numbers of ganglia assayed for each group are in parentheses. Bars represent mean ± standard error of the mean for each group.

(33). HSV DNA levels were similar in ganglia latently infected with wt ($[1 \pm 0.2] \times 10^4$ molecules/ganglion) and *dl*LAT1.8 ($[8 \pm 1] \times 10^3$ molecules/ganglion) and just slightly lower in ganglia latently infected with FSLAT$^+$ ($[4 \pm 1] \times 10^3$ molecules/ganglion). The finding of similar amounts of viral DNA in ganglia latently infected with wt or *dl*LAT1.8 is consistent with results of most studies using the same or different LAT deletion mutants (2, 12, 19, 36, 46, 55, 68). Quantification of the major LATs using primer LAT-2 for reverse transcription and the LAT-1–LAT-2 pair for PCR (Fig. 1) revealed 10^5 to 10^6 molecules per viral genome in wt or FSLAT$^+$-infected ganglia (Fig. 3A). In contrast, in *dl*LAT1.8-infected ganglia, we measured an average of only five molecules of RNA per genome from this region (Fig. 3A). Also, the amount of RNA was much more variable from ganglion to ganglion, with two-thirds of the values for *dl*LAT1.8-infected ganglia being spread over a >100-fold range, while two-thirds of the values for wt-infected ganglia were clustered within a 3-fold range (11). Thus, the deletion in *dl*LAT1.8 decreases the abundance of the major LATs by at least 5 orders of magnitude.

The LAT deletion results in increased *ICP4* transcripts. To follow up our previous preliminary finding of increased accumulation of *ICP4* transcripts in ganglia latently infected with *dl*LAT1.8 (33), we assayed for these transcripts in a large number of ganglia infected with either wt, *dl*LAT1.8, or FSLAT$^+$. Consistent with our previous findings (33), levels of *ICP4* RNA in wt-infected ganglia were highly variable (two-thirds of the values spread over a >100-fold range), but in 12 of 47 ganglia (26%), these levels were equal to or greater than one molecule per viral genome. The average *ICP4* RNA level per viral genome (arithmetic mean) in *dl*LAT1.8-infected ganglia (six molecules per genome) was ~5-fold greater than that in wt-infected ganglia (Fig. 3B and 4A). This difference is statistically significant ($P = 0.038$, Student's t test). Similarly the average amount of *ICP4* RNA per ganglion was substantially greater (~8-fold) in *dl*LAT1.8-infected ganglia than in wt-infected ganglia (11). In addition, a greater proportion (20 of 45 [44%]) of *dl*LAT1.8-infected ganglia had levels of *ICP4* RNA that exceeded one molecule per genome than did wt-infected ganglia ($P < 0.05$, Fisher's exact test). The average *ICP4* RNA level in FSLAT$^+$-infected ganglia was similar to that in wt-infected ganglia (Fig. 3B) and significantly different from that in *dl*LAT1.8-infected ganglia ($P = 0.05$, Student's t test). Thus, the LAT deletion results in increased accumulation of transcripts from the *ICP4* gene.

Effects of the LAT deletion on *tk* transcripts. We next measured levels of RNA specific to the *tk* gene, which is an E gene whose expression stringently depends on ICP4 (18, 47, 49) yet is distant from the *ICP4* gene and from the LAT locus. In accordance with our results with *ICP4* RNA, we found that the average amount of *tk* RNA (70 molecules/viral genome) in *dl*LAT1.8-infected ganglia was more than 10-fold greater than that detected in wt- and FSLAT$^+$-infected ganglia (Fig. 3C and 4B). This difference was statistically significant ($P = 0.016$, Student's t test). Thus, the LAT deletion increases expression of *ICP4* and *tk* transcripts.

We had previously observed that the amounts of *tk*-specific RNA and *ICP4*-specific RNA correlated in five individual ganglia latently infected with either *dl*LAT1.8 or KOS (33). We investigated whether this correlation would pertain when a much larger set of ganglia was examined and whether the LAT deletion would affect it. We found that the correlation remained strong ($r^2 = 0.69$) for *dl*LAT1.8-infected ganglia (Fig. 5A) but was not apparent ($r^2 = 0.07$) for KOS-infected ganglia (Fig. 5B) or for FSLAT$^+$-infected ganglia ($r^2 = 0.002$ [data not shown]). Thus, the LAT deletion positively affects the correlation between levels of *ICP4* and *tk* transcripts.

The LAT deletion decreases transcripts antisense to *ICP4*. In the course of our studies of *ICP4* transcripts in latently infected ganglia (33), we preliminarily observed transcripts antisense to *ICP4* transcripts (Anti-4) in these ganglia (34). We wondered what the effect of the LAT deletion would be on Anti-4 transcripts, as they would be encoded downstream from and on the same strand as highly abundant LAT species (Fig. 1). To address this question, we established a quantitative assay for Anti-4 transcripts (see Materials and Methods) and applied this assay to investigate the effect of the LAT deletion on Anti-4 transcript accumulation (Fig. 4C). In wt-infected ganglia, on average, 75 Anti-4 RNA molecules per viral genome were detected (Fig. 3D), in substantial excess to the average amount of *ICP4* transcripts detected (Fig. 3B). In *dl*LAT1.8-infected ganglia, the average number of Anti-4 molecules per genome was 15-fold lower (~5 molecules per genome [Fig. 3D]). This represents a statistically significant difference ($P < 0.01$, Student's t test) compared with wt. Rescue of the LAT deletion in FSLAT$^+$ restored the higher level of Anti-4 RNA (significantly different from *dl*LAT1.8 value, $P < 0.01$). Thus, the *dl*LAT1.8 deletion substantially decreased the level of Anti-4 transcripts while increasing the levels of *ICP4* and *tk* transcripts in latently infected ganglia.

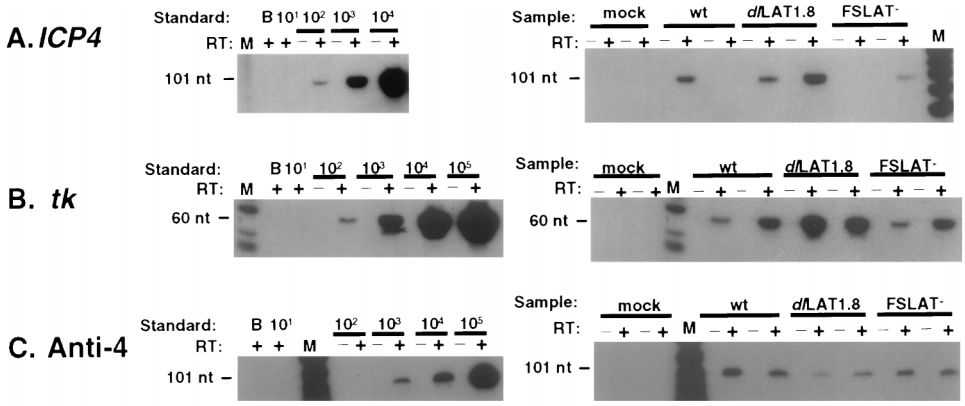

FIG. 4. HSV-specific RNAs in latently infected ganglia at 30 days postinoculation. Autoradiograms of probed blots of *ICP4* (A), *tk* (B), and Anti-4 (C) RT-PCR products separated on polyacrylamide gels are shown. The left portion of each panel shows the results obtained with the indicated numbers of synthetic RNA molecules (Standard). B, water blank. The right portion of each panel shows the results obtained with individual ganglion RNAs. RNAs were reacted without (−) or with (+) reverse transcriptase (RT), and the products are displayed in adjacent lanes. mock, ganglia from animals inoculated with media not containing virus; wt, ganglia from animals inoculated with KOS; *dl*LAT1.8, ganglia from animals inoculated with *dl*LAT1.8; FSLAT$^+$, ganglia from animals inoculated with FSLAT$^+$; M, molecular weight marker. The results in the different panels derive from different ganglia; e.g., results from six different KOS-infected ganglia are shown. The sizes of the RT-PCR products are indicated to the left of each autoradiogram. nt, nucleotides.

DISCUSSION

In this report, we show that a deletion in the HSV-1 LAT locus results in increases in the accumulation of transcripts from the *ICP4* and *tk* genes and decreases in accumulation of transcripts antisense to *ICP4* RNA in latently infected mouse ganglia. We will first discuss possible provenances of the antisense transcripts and then discuss possible implications of the effects of the LAT mutation on viral gene expression.

A transcript antisense to *ICP4* RNA. We have detected a transcript antisense to *ICP4* RNA, Anti-4, in latently infected ganglia. Because the deletion in *dl*LAT1.8, which removes the LAT promoter, decreases Anti-4 expression substantially, the simplest explanation for the provenance of most Anti-4 molecules is that they initiate at the LAT promoter and extend beyond the putative LAT polyadenylation signal to cross the region corresponding to the 4-1, 4-2 primer pair (Fig. 1). Assuming that the ~8-kb minor LAT species that has been detected by in situ hybridization and Northern blot hybridization (16, 17, 20, 42, 43, 51, 69) is ~1% as abundant as the major LAT species, even ~1 to 2% readthrough of this minor LAT species past the putative LAT polyadenylation signal could give rise to long transcripts that would account for the number of Anti-4 molecules observed in wt-infected ganglia. (By this explanation, the Anti-4 molecules observed in *dl*LAT1.8-infected ganglia would arise from a promoter other than the LAT promoter.) Thus, most Anti-4 molecules could be long LAT species that are 0.01 to 0.02% as abundant as the major LATs. This would be akin to the long LATs encoded by Marek's disease virus that are antisense to transcripts encoding a homolog of ICP4 recently reported by Cantello et al. (9). HSV LAT species antisense to the 3′ end of *ICP4* RNA have been detected during HSV latency by in situ hybridization (43), but none has yet been reported to extend across the sequences used to detect Anti-4.

Alternatively, it is also possible that some (e.g., the ~5 Anti-4 RNA molecules per viral genome expressed by *dl*-LAT1.8) or all Anti-4 RNA corresponds to a family of transcripts known as L/STs (66) or other transcripts which are controlled by promoters αX, βX (4, 5), or as yet unrecognized transcripts. Perhaps LAT expression up-regulates L/ST expression in a manner similar to that proposed for activation of adenovirus E1b expression by E1a transcription (45). Regardless of its provenance, Anti-4 expression, like expression of the LATs (33), varies relatively little from ganglion to ganglion in mice infected with LAT$^+$ viruses (11), and Anti-4 can be considered a transcript that is expressed consistently at low levels in most latently infected ganglia.

A viral function represses accumulation of transcripts from productive-cycle genes in latently infected ganglia. Our results show that a viral function that is affected by a LAT deletion represses the accumulation of transcripts from at least two productive cycle genes, *ICP4* and *tk*, in mouse trigeminal ganglia latently infected with HSV. As previously discussed (33), we do not know whether these transcripts are identical to the *ICP4* and *tk* transcripts prevalent in productively infected cells

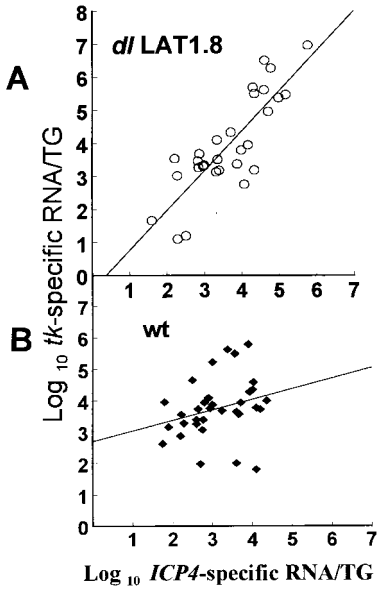

FIG. 5. Correlation of expression of *ICP4* RNA and *tk* RNA in ganglia latently infected with *dl*LAT1.8 (A) or with KOS (B). The amount of *tk* RNA in each trigeminal ganglion (TG) was plotted against the amount of *ICP4* RNA in the same trigeminal ganglion. The best-fit lines were generated by linear regression of $\log_{10}$ values.

or whether those transcripts are translated into active protein. However, the available evidence increasingly points in that direction. In at least 50% of ganglia tested, most of the *ICP4* transcripts initiate in a manner consistent with utilization of the *ICP4* promoter (11). Similarly, preliminary data indicate that most *tk* transcripts initiate in a promoter-specific manner in some ganglia (32). Here, we show that a viral mutation increases the abundance of *ICP4* and *tk* transcripts and positively affects the correlation between *ICP4* and *tk* transcripts. These findings argue against the correlation being due to a general increase in cellular transcriptional activity. Rather, we favor the interpretation that in *dl*LAT1.8-infected ganglia, *ICP4* transcripts encode active ICP4 that induces *tk* expression and that the LAT deletion relieves repression of at least one IE and one E gene.

The *ICP4* and *tk* transcripts that we observe could be due to expression from truly latent genomes or from genomes that are undergoing abortive or full-blown reactivation and thus perhaps viral replication. In the first case, our results would indicate that a viral function represses expression of these transcripts during latency. In the second case (although it is difficult to distinguish between abortive reactivation and a less repressed form of latency), our results would indicate that a viral function represses expression during reactivation. In either case, inasmuch as latency entails repression of productive-cycle gene expression, our results imply that HSV encodes a function that helps to maintain latency.

It is important to emphasize that even in *dl*LAT1.8-infected ganglia, ~30% of ganglia expressed very low levels of *ICP4* RNA (<0.1 molecule per viral genome [11]). Moreover, the LAT deletion is not sufficient to prevent the establishment of latency. Thus, repression of productive gene expression by the function affected by the LAT deletion is not the only mechanism for maintaining latency; other factors, perhaps both viral and cellular, are likely to be involved.

The repressive function maps to the LAT locus. The 1.8-kbp deletion in *dl*LAT1.8 removes 1 kbp of LAT coding sequences and 0.8 kbp of upstream sequences (36), including sequences known to be important for LAT promoter function (22, 23). Although it is possible that the repression of productive-cycle gene expression alleviated by this deletion is unrelated to the LATs (e.g., via effects on low-abundance L transcripts that enter this region [57]), a more attractive model is that repression is mediated by the LATs. Within this framework, our data provide direct evidence for a role of the LATs in maintenance of latency, as originally suggested (62). Thus, one function of the LATs may be to restrain productive-cycle gene expression in neurons. This concept is independently supported by findings during acute infection using in situ hybridization analyses in which a similar LAT deletion caused fourfold increases in the number of neurons expressing productive-cycle RNAs (26). A role for the LATs in repressing productive-cycle gene expression is particularly appealing because it would provide a direct mechanistic link between the productive and latent programs of gene expression. Interestingly, there is evidence that the major activator of productive gene expression, ICP4, can repress the LAT promoter (1, 50); thus, it may be that a balance between ICP4 and the LATs helps to set the latent-productive switch. This idea and the involvement of other IE genes in this switch are currently being investigated.

Potential mechanisms for LAT-mediated repression. Assuming that the LATs mediate repression of productive-cycle gene expression in neurons, there are several possible mechanisms for how this could occur. The LATs could encode proteins that serve as repressors. If a protein is involved, we would postulate that it not only decreases the accumulation of *ICP4* transcripts but also decreases the likelihood that *ICP4* transcripts result in a protein that induce *tk* expression. This could occur by preventing expression of a cofactor for ICP4-mediated induction. Interestingly, it has recently been suggested that a protein encoded by L/STs, which theoretically could also be encoded by certain LAT species, represses ICP0 expression by inhibiting splicing (6). If so, this could lead to a failure of ICP0 to stimulate expression of *ICP4* and other HSV genes. A second possible mechanism for LAT-mediated repression stems from our observation that in wt-infected ganglia, Anti-4 RNA was in vast excess to *ICP4* RNA both on average (Fig. 2) and in individual ganglia (11). This finding and the inverse effects of the LAT deletion on *ICP4* and Anti-4 RNA levels suggest the possibility of antisense regulation of the abundance and/or translation of *ICP4* transcripts. The magnitudes of the average decrease in Anti-4 (15-fold) and the average increase in *ICP4* transcripts due to the LAT deletion (5-fold) are nearly identical to that observed in another potential example of antisense regulation in HSV, where promoter mutations that reduce *tk* transcripts 10- to 20-fold result in a 6-fold increase in certain *UL24* transcripts (15). The effect of the LAT deletion on the correlation between the levels of *ICP4* and *tk* transcripts is consistent with Anti-4 also suppressing the translation of ICP4, thereby decreasing induction of *tk* expression.

Alternatively, other, less direct mechanisms, for example, antisense repression of *ICP0* (24, 62), which could in turn lead to a failure of ICP0 to induce expression of *ICP4* and other genes, are possible. These various mechanisms are not mutually exclusive; for example, there is evidence that Epstein-Barr virus latency is maintained by at least two different strategies: blockade of signal transduction by a viral protein and negative regulation of an IE gene by an antisense transcript (41, 48). Indeed, as discussed above, it seems likely that multiple mechanisms of repression are involved in maintaining HSV latency.

Relationship of repression to reactivation. Mutant *dl*LAT1.8 and most other LAT-null mutants replicate as efficiently as their respective wt viruses in cell culture and at peripheral sites and in ganglia of experimental animals (2, 19, 36, 46, 55, 61, 68). However, certain LAT mutants, including *dl*LAT1.8, exhibit reduced reactivation frequencies following explant of latently infected ganglia from mice and substantially reduced reactivation in vivo in rabbits and guinea pigs (2, 3, 19, 28, 29, 36, 46, 61, 67, 68). How can this phenotype be reconciled with the increases in productive gene expression that we have observed in latently infected ganglia? Naively, one might expect to observe a greater degree of reactivation and even spontaneous reactivation with less restraint on the productive program rather than a reduction in reactivation frequency.

If one assumes that both the reactivation and gene expression phenotypes are mediated by the LATs, one possible explanation is that the LATs have at least two roles: repression of productive-cycle gene expression in ganglia and enhancement of reactivation following an appropriate stimulus. For this latter role, the structure of the 5′ portion of the LAT coding region may act to prime reactivation, as suggested by Bloom et al. (3). A second possibility, which would not require multiple roles for the LATs, is that they repress expression of productive-cycle genes that would otherwise elicit infiltration of immune cells that could clear infected cells or release cytokines, such as gamma interferon (10, 39), which might then reduce reactivation frequency. Such immune responses, normally dampened by the LATs, could occur during the acute phase of ganglionic infection or, possibly, in latently infected ganglia. This might explain why *dl*LAT1.8 exhibited a reactivation defect when relatively intact ganglia were assayed but not when the ganglia were dissociated into individual cells (36, 38). A

third possibility is that the LATs repress expression of certain viral activities that could otherwise damage cells, leaving them unable to support reactivation. Expression of these activities could occur either during the latent phase of infection or, as documented by Garber et al. (26), during acute infection. Regardless, a full understanding of LAT function will require an understanding of the effects of mutations such as the *dl*LAT1.8 deletion on both gene expression and reactivation. Further analyses of the mechanisms by which the virus acts to repress expression of productive-cycle genes should contribute to a better understanding of latency and may aid in developing strategies to defeat recurrent herpetic disease.

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The first two authors contributed equally to this study.

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