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Different Mechanisms Regulate Productive Herpes Simplex Virus 1 (HSV-1) and HSV-2 Infections in Adult Trigeminal Neurons

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Herpes simplex virus 1 (HSV-1) and HSV-2 establish latency in different neuronal subtypes (A5+ and KH10+) in murine trigeminal ganglia, results which correlate with restricted productive infection in these neurons *in vitro*. HSV-2 latency-associated transcript (LAT) contains a *cis*-acting regulatory element near the transcription start site that promotes productive infection in A5+ neurons and a second element in exon 1 that inhibits productive infection in KH10+ neurons. HSV-1 contains no such regulatory sequences, demonstrating different mechanisms for regulating productive HSV infection in neurons.

Herpes simplex virus 1 (HSV-1) and HSV-2 express productive cycle genes in some neurons and establish latent infection in others. We previously demonstrated that HSV-1 and HSV-2 preferentially establish latency in different neuronal subtypes in murine sensory ganglia: HSV-1 in A5+ neurons and HSV-2 in KH10+ neurons (1, 2). In dissociated adult murine trigeminal cultures (AMTC), we previously found that HSV-1 productively infected KH10+ but not A5+ neurons while HSV-2 productively infected A5+ but not KH10+ neurons (Fig. 1A) (3). Thus, the neuronal subtypes that restrict productive HSV infection *in vitro* are the same neurons that harbor latent infection *in vivo*. Both host and viral factors may play roles in regulating neuronal specificity (1, 2, 4–7). To determine if the latency-associated transcript (LAT) region regulates productive infection in A5+ and KH10+ neurons, we assayed HSV infection of AMTC using a series of LAT deletion and chimeric viruses (5).

The HSV-2 LAT region contains two regulatory sequences for productive infection of neurons. HSV-2 (333) productively infects 36% of A5+ neurons in AMTC but only 3% of KH10+ neurons (Fig. 1B). In contrast, dLAT (NotI-NotI deletion of the HSV-2 LAT promoter) (Fig. 1D) (8) productively infects only 5% of A5+ neurons (Fig. 1B). The LAP2 virus (LAT promoter 2 deletion in HSV-2 exon 1) (Fig. 1D) (9) productively infects A5+ and KH10+ neurons equivalently (39% and 36%, respectively) (Fig. 1B). Thus, two distinct functional elements within HSV-2 LAT regulate productive infection: the NotI-NotI region promotes productive infection in A5+ neurons, and the LAP2 region inhibits productive infection in KH10+ neurons.

HSV-2/LAT1 is a chimera in which 2.8 kb of the HSV-2 LAT region (promoter, exon 1, and ~1.5 kb of the intron, excluding ICP0 coding sequences) was replaced by the same region from HSV-1 (10). HSV-2/LAT1 preferentially establishes latency in A5+ neurons *in vivo*, suggesting that HSV-1 LAT in the context of HSV-2 changes the viral phenotype to that of HSV-1 (1). In AMTC, HSV-2/LAT1 productively infects 22% of KH10+ neurons but only 4% of A5+ neurons, in contrast to HSV-2 or the rescuant, HSV-2/LAT1-R (Fig. 1C). Thus, the pattern of productive infection with HSV-2/LAT1 resembles that of HSV-1, with productive infection in KH10+ neurons but restricted infection in A5+ neurons. Since the HSV-1 LAT region in HSV-2/LAT1 replaces sequences that were deleted in dLAT and LAP2, the observed phenotype of HSV-2/LAT1 might be due to either deletions of HSV-2 LAT or inserted HSV-1 LAT-associated functions. To distinguish between these possibilities, we studied the effect of HSV-1 LAT deletions on the pattern of productive infection in AMTC. Unlike HSV-2, HSV-1 LAT mutations involving the promoter, exon 1, and the intron had no effect on the neuronal pattern of infection in AMTC compared to wild-type HSV-1 (Fig. 2A).

HSV-1/LAT2, the reciprocal of HSV-2/LAT1 (Fig. 2C) (11), has a latent phenotype similar to that of wild-type (WT) HSV-2 *in vivo* (6). In AMTC, HSV-1/LAT2 productively infects A5+ and KH10+ neurons equivalently (30% and 31%, respectively), in contrast to HSV-1 and the rescuant, which infect 3% of A5+ neurons and 22% of KH10+ neurons (Fig. 2B). Thus, only the function that promotes productive infection in A5+ neurons was transferred from HSV-2 to HSV-1. This suggests that the HSV-2 LAT NotI-NotI region, which promotes productive infection in A5+ neurons, contains a transferrable *cis*-acting element, while the HSV-2 LAP2 region, which contains a function that restricts productive infection in KH10+ neurons, is not transferrable to HSV-1 as an independent functional element.

We hypothesized that the HSV-2 LAP2 sequence requires an additional factor to maintain functionality in the context of HSV-1. Therefore, we coinfected AMTC with HSV-1/LAT2 and HSV-2-green fluorescent protein (GFP) (12) and assayed the pattern of productive infection of each virus. Only 5% of KH10+ neurons were productively infected with HSV-1/LAT2, similar to percentages with either HSV-2 or HSV-2-GFP alone (Fig. 1 and 2B), in contrast to HSV-1 or HSV-1/LAT2 alone (Fig. 2B). Thus, either a viral factor from outside the HSV-2 LAT region or a cellular factor produced during HSV-2 infection is required for the

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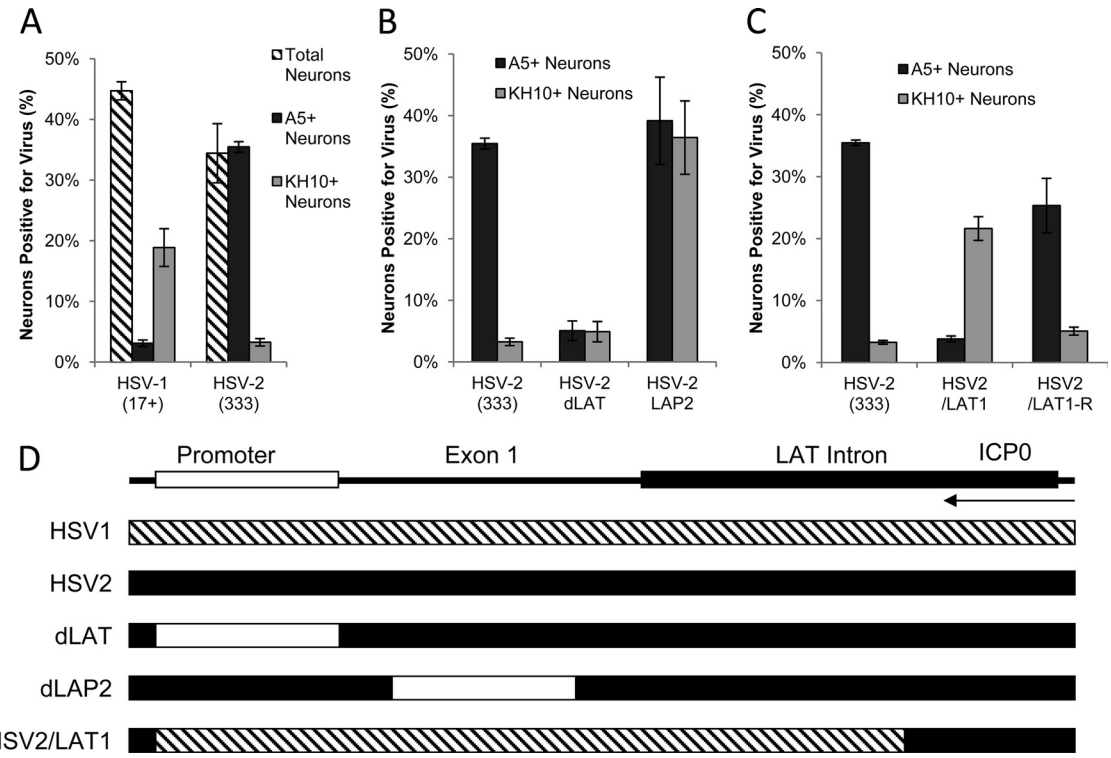

FIG 1 Productive infection of cultured primary adult murine TG neurons with HSV-2 LAT region deletion and chimeric viruses (multiplicity of infection [MOI], 30; 10 h postinoculation). (A) Percentages of total (hatched bars), A5+ (black bars), and KH10+ (gray bars) neurons productively infected with HSV-1 and HSV-2, as identified by MAbs A5 and KH10, and polyclonal antisera against HSV-1 or HSV-2. (These data were published previously [3] and are shown here for reference). (B) Percentages of A5+ and KH10+ neurons productively infected with HSV-2 wild type (333) and HSV-2 LAT region deletion viruses (dLAT and LAP2). (C) Percentages of A5+ and KH10+ neurons productively infected with HSV-2 wild type (333), chimeric virus HSV2/LAT1, and its rescuant, HSV2/LAT1-R. (D) Maps illustrating HSV-2 LAT region deletion and chimeric sequence swap. Deletions are indicated by white boxes on the HSV-2 LAT region (black), and the HSV-1 sequence placed into HSV-2 is indicated by a hatched box on the HSV-2 background (black). ICP0 transcript is shown for reference.

HSV-2 LAP2 region to restrict productive infection in KH10+ neurons.

Mapping regulatory domains in LAT regions. We next used previously characterized HSV-2/HSV-1 chimeras (P1, S1, and E1) containing HSV-1 (17+) LAT regions in an HSV-2 (333) background (Fig. 3B) (4, 13) to map functional domains of the LAT region that regulate productive infection in A5+ and KH10+ neurons. In P1, the HSV-1 LAT promoter (NotI-PvuI) drives expression of HSV-2 LAT (13). In S1, the HSV-2 LAT promoter drives expression of HSV-1 LAT (PvuI-XhoI) (13). The PvuI site is just downstream of the HSV LAT TATA box (Fig. 4). P1 productively infected only 1.6% of A5+ neurons (Fig. 3A), similar to dLAT (Fig. 1B), while S1 productively infected A5+ and KH10+ neurons equivalently (19% and 22%, respectively), similar to LAP2 (Fig. 1B). These results suggest that transfer of HSV-1 LAT sequences into HSV-2 does not transfer a function from HSV-1 to HSV-2 but simply deletes the functional regions from HSV-2 that stimulate productive infection in A5+ neurons (NotI-PvuI) or inhibit productive infection in KH10+ neurons (LAP2). Thus, studies with chimeric viruses must be carefully interpreted since functional elements can be both added and deleted during viral construction.

E1, in which the HSV-2 LAT region from TATA to the intron 5′ splice site was replaced by the same region from HSV-1 (exon 1) (4), produced a similar pattern of infection in AMTC as HSV-1, with productive infection of KH10+ neurons (22%) but minimal productive infection in A5+ neurons (2%). Thus, transfer of HSV-1 LAT exon 1 into HSV-2 effectively deletes the regions responsible for both productive infection of A5+ neurons and restricted infection of KH10+ neurons. Sequence analysis suggests that the HSV-2 function that promotes productive infection in A5+ neurons maps between the LAT TATA box and the PvuI site 44 bp downstream. This region contains multiple putative regulatory sequences and is nearly identical in HSV-1 and HSV-2 except for an apparent 20-bp deletion in HSV-1 (Fig. 4). We conclude that these 20 bp are required for productive HSV infection of A5+ neurons since viruses containing them productively infect A5+ neurons (HSV-2, HSV-1/LAT2, and S1), and those lacking them (HSV1, HSV-2/LAT1, P1, and E1) are restricted for productive infection in A5+ neurons.

HSV-1 and HSV-2 productive infections are regulated by different mechanisms in neurons. Monoclonal antibodies (MAbs) A5+ and KH10+ recognize two distinct populations of murine nociceptive neurons. Nerve growth factor (NGF)-responsive A5+ neurons are largely calcitonin gene-related peptide (CGRP) positive with Aδ fibers, while glial cell line-derived neurotrophic factor (GDNF)-responsive KH10+ C-fiber neurons colabel with isolectin-B4 (IB4) (2, 14, 15). Similar, but not identical, Aδ and C-fiber neuronal populations are found in human sensory ganglia and innervating human skin (16, 17). A5+ neurons restrict productive HSV-1 infection and are the major site of HSV-1 latency. KH10+ neurons restrict productive HSV-2 infection and are the

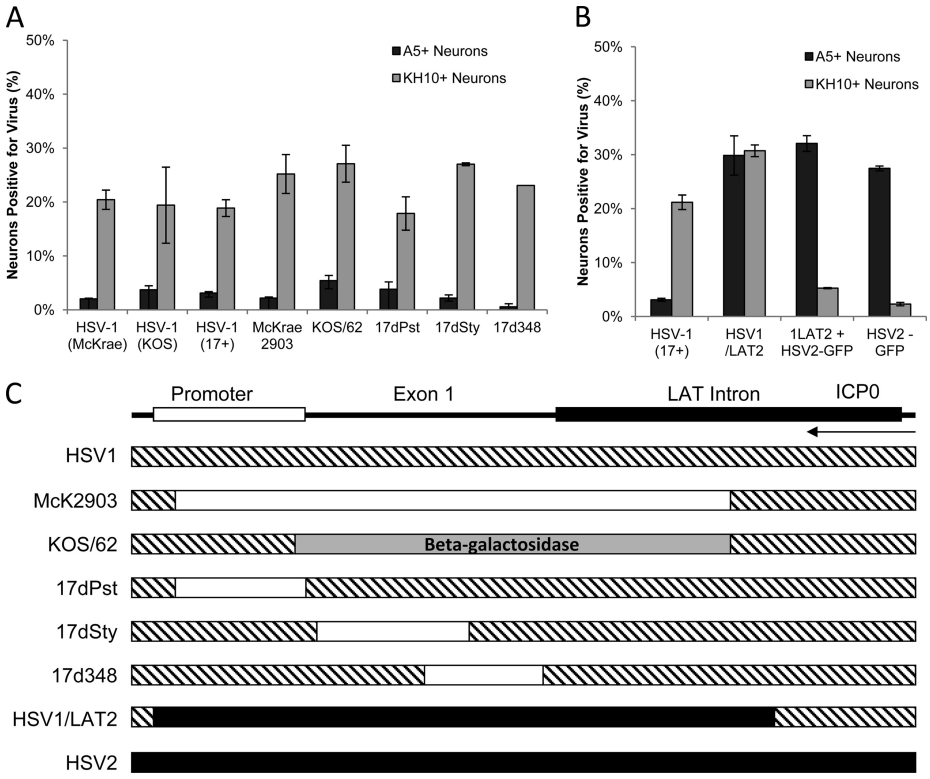

FIG 2 Productive infection of cultured primary adult murine TG neurons with HSV-1 LAT region deletion and chimeric viruses (MOI, 30; 10 h postinoculation). (A) Percentages of A5+ and KH10+ neurons productively infected with HSV-1 wild-type (McKrae, KOS, 17+) and HSV-1 LAT region deletion (McKrae 2903, KOS/62, 17dPst, 17dSty, 17d348) viruses. (B) Percentages of A5+ and KH10+ neurons productively infected with HSV-1, chimeric virus HSV1/LAT2, or HSV2-GFP or coinfected with both HSV1/LAT2 and HSV2-GFP, evaluated for productive infection using polyclonal antisera to detect HSV-1 and HSV1/LAT2 and GFP expression to detect HSV2-GFP. (C) Maps illustrating HSV-1 LAT region deletions and chimeric sequence swap. Deletions are indicated by white boxes, and beta-galactosidase is indicated by a gray box on the HSV-1 LAT region (hatched); HSV-2 sequence placed into HSV-1 is indicated by a black box on the HSV-1 background (hatched). ICP0 transcript is shown for reference.

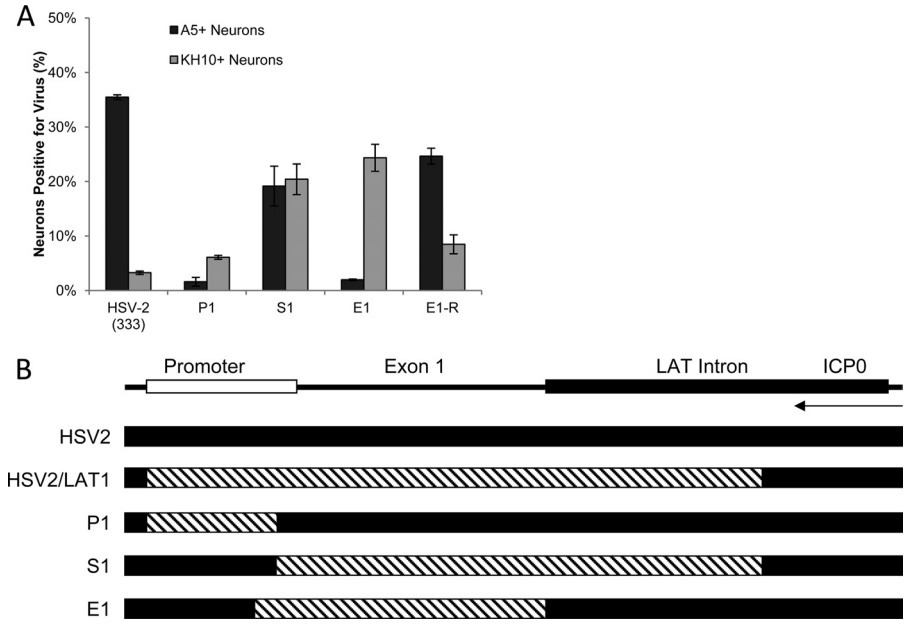

FIG 3 Productive infection of cultured primary adult murine TG neurons with HSV-2, the chimeric viruses P1, S1, and E1, and the E1 rescuant (MOI, 30; 10 h postinoculation). (A) Percentages of A5+ and KH10+ neurons productively infected with HSV-2 (333), the chimeric viruses P1, S1, and E1, and the E1 rescuant E1-R. (B) Maps illustrating LAT region chimeric sequence swaps. HSV-1 LAT sequences (hatched) replaced HSV-2 LAT sequences (black). P1 contains the HSV-1 LAT promoter from NotI to the PvuI site just downstream of the TATA box (the 5′ end of the region replaced in HSV2/LAT1); S1 contains the HSV-1 LAT sequence from the PvuI site through ~1.5 kb downstream of the 5′ splice site of the intron (the 3′ end of the region replaced in HSV2/LAT1) but excludes ICP0 coding sequences; E1 contains HSV-1 LAT exon 1 from the TATA box to the 5′ splice site of the intron. ICP0 transcript is shown for reference.

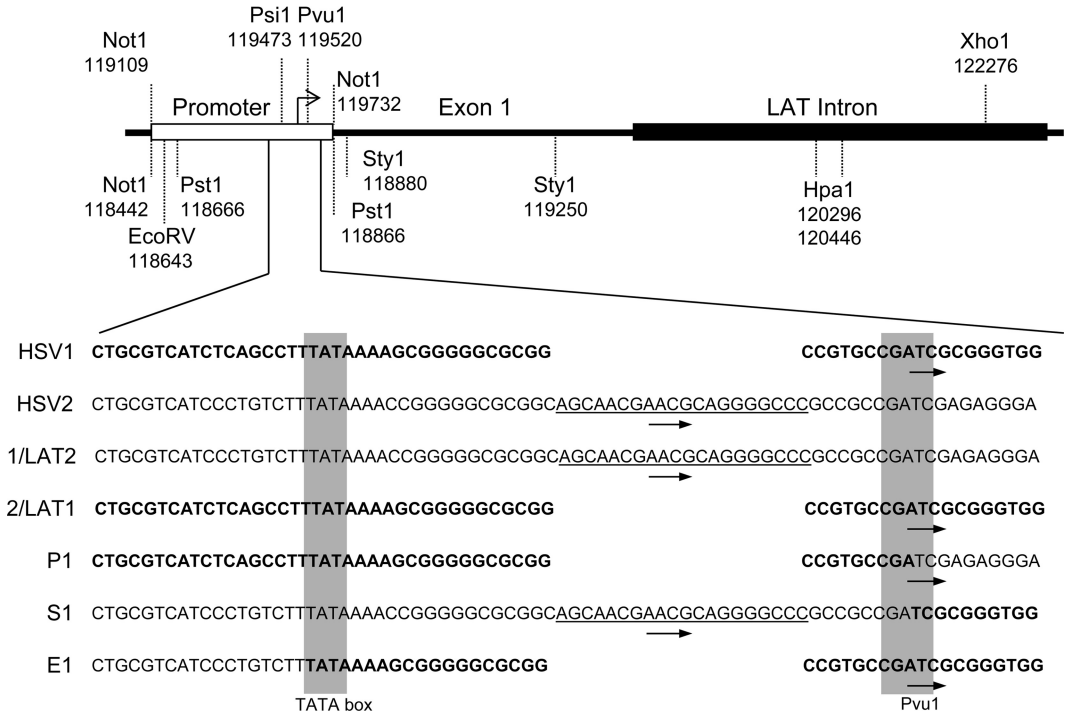

FIG 4 Alignment of HSV-1, HSV-2, and chimeric viruses. Sequences of HSV-1, HSV-2, HSV1/LAT2, HSV2/LAT1, P1, S1, and E1 are aligned at the TATA box and the PvuI restriction enzyme site just downstream (gray boxes). HSV-1 sequences are bold, and HSV-2 sequences are normal font. Arrows indicate LAT transcription start sites. Underlined sequences are HSV-2 sequences present in wild-type HSV-2, HSV1/LAT2, and S1; this sequence is necessary for productive infection in A5+ neurons.

major site of HSV-2 latency. Our studies demonstrate that the mechanisms that regulate these phenotypes are mediated in part by two regulatory elements in the HSV-2 LAT region: a 20-bp sequence near the TATA box contains a *cis*-acting element that promotes productive infection in A5+ neurons, while LAP2 inhibits productive infection in KH10+ neurons but requires an additional factor. HSV-1 LAT contains no such regulatory elements, demonstrating that HSV-1 and HSV-2 neuron specificity is regulated by different mechanisms.

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