Truncation of the C-Terminal Acidic Transcriptional Activation Domain of Herpes Simplex Virus VP16 Produces a Phenotype Similar to That of the \textit{in}1814 Linker Insertion Mutation

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Herpes simplex virus (HSV) virions contain one or more factors that stimulate transcription of the viral immediate-early (IE) genes (27). Early studies demonstrated that the activity resides in the virion tegument (3) and that the major tegument protein VP16 activates IE promoters in transient cotransfection assays (7, 8). Intensive biochemical and molecular studies have since clarified the mechanism of action of VP16 as a transcriptional activator (reviewed in references 12 and 35); it bears a strong C-terminal acidic transcriptional activation domain (30, 36) and is targeted to the TAATGARATTC consensus sequence in IE promoters through interactions with the cellular proteins Oct1 and HCF (4, 5, 10, 11, 14–19, 21, 22, 24, 25, 28, 29, 33, 38, 39). VP16 also plays an essential but not yet defined role in virion assembly (1, 37) and binds to at least two other tegument proteins, the virion host shutoff protein vhs (31) and VP22 (9).

It seems plausible that the VP16 molecules delivered by the infecting virus particle play an important role in triggering the onset of IE transcription during HSV infection. Supporting this view, Preston and colleagues showed that a linker insertion mutation that disrupts the promoter-targeting function of VP16 (in1814) results in a greatly increased particle-to-PFU ratio in plaque assays and reduced levels of expression of some IE genes during infection at relatively high multiplicities (1, 2). The defect in plaque formation exhibited by in1814 is at least partially complemented in U2OS osteosarcoma cells, which had previously been shown to complement ICP0 null mutations. Taken in combination, these data confirm the key role of VP16 in triggering the onset of the HSV lytic cycle.

We examined the phenotype of a herpes simplex virus (HSV) type 1 mutant (V422) in which the C-terminal acidic activation domain of the virion transactivator VP16 is truncated at residue 422. The efficiency of plaque formation by V422 on Vero cells was boosted by approximately 100-fold by including hexamethylene bis-acetimide (HMBA) in the growth medium, as previously observed with the in1814 VP16 linker insertion mutant isolated by Preston and colleagues. V422 displayed severely reduced levels of the immediate-early transcripts encoding ICP0 and ICP4 during infection in the presence of cycloheximide, and this defect was partially overcome by the addition of HMBA. The defect in plaque formation exhibited by V422 and in1814 was efficiently complemented in U2OS osteosarcoma cells, which had previously been shown to complement ICP0 null mutations. Taken together, these data confirm the key role of VP16 in triggering the onset of the HSV lytic cycle.

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TABLE 1. Effects of HMBA and cell type on plaquing efficiency of VP16 and ICP0 mutants

<table>
<thead>
<tr>
<th>Virus</th>
<th>Titer with the following cell lines:</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vero</td>
<td>Vero + HMBA</td>
<td>U2OS</td>
<td>U2OS + HMBA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V422</td>
<td>2.6 x 10^7</td>
<td>2.2 x 10^7</td>
<td>6.4 x 10^7</td>
<td>1.4 x 10^8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8MAR</td>
<td>7.6 x 10^8</td>
<td>8.6 x 10^8</td>
<td>3.4 x 10^9</td>
<td>4.2 x 10^9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in1814</td>
<td>1.0 x 10^9</td>
<td>9.8 x 10^8</td>
<td>7.6 x 10^8</td>
<td>2.2 x 10^8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in1814R</td>
<td>2.6 x 10^8</td>
<td>2.8 x 10^8</td>
<td>1.5 x 10^8</td>
<td>1.5 x 10^8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n212 (ICP0⁻)</td>
<td>5.8 x 10^6</td>
<td>5.0 x 10^6</td>
<td>2.0 x 10^6</td>
<td>1.8 x 10^6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The titers of virus stocks were determined by plaque assays on Vero and U2OS cells in the presence and absence of 3 mM HMBA. The V422 and in1814 stocks were produced on Vero cells in the presence of HMBA, and n212 was grown on U2OS cells.

plaque formation by V422 and in1814 in the absence of HMBA (Table 1). Both mutants produced substantially more plaques on U2OS cells than on Vero cells; in contrast, the corresponding wild-type strains exhibited a twofold-lower titer on U2OS cells. The increase in apparent titer observed with U2OS cells was similar to that induced by HMBA in Vero cells (ca. 100-fold) and comparable to that observed with the ICP0-deficient mutant n212 (6). Moreover, HMBA had only a relatively small stimulatory effect on the VP16 mutants in U2OS cells (ca. twofold). Thus, U2OS cells complement the VP16 mutants to approximately the same degree to which they complement an ICP0 mutant in a plaque assay. Although the mechanism of this complementation is unclear, one interpretation is that the cellular ICP0-like function proposed by Yao and Schaffer (40) partially bypasses the requirement for VP16 activation function, perhaps by facilitating IE gene expression at low multiplicities of infection.

We examined the effect of the V422 mutation on accumulation of the IE transcripts encoding ICP4 and ICP0 during infection of Vero and U2OS cells. In order to eliminate the potentially confounding effects of ICP4-mediated repression of IE transcription and differences in rates of progression through the lytic cycle, the assay was conducted in the presence of cycloheximide to prevent viral protein synthesis. Cells were infected with 10 PFU of V422 per cell (titer determined on Vero cells in the presence of HMBA) and 8MAR in the presence and absence of 8 mM HMBA, and total cellular RNA harvested at 6 h postinfection was analyzed by primer extension and differences in rates of progression through the lytic cycle, the assay was conducted in the presence of cycloheximide to prevent viral protein synthesis. Cells were infected with 10 PFU of V422 or 8MAR per cell in the presence of cycloheximide, and ICP0 and ICP4 transcript levels were examined by primer extension (Fig. 1). Signal intensities were then quantified by PhosphorImager analysis, and the results for each mutant were expressed as fold reduction relative to the corresponding wild-type strain (Table 2). The results demonstrate that in1814 displays a readily detectable defect in the accumulation of both ICP0 and ICP4 transcripts during infection of Vero cells in the presence of cycloheximide. We observed substantial variation in the wild-type-to-mutant ratios with each mutant over the course of three experiments (Table 2). However, in each experiment V422 displayed a defect greater than that of in1814. It is not clear whether this apparent difference stems from variation between HSV1 strains (KOS versus 17) or from the predicted ability of the V422 polypeptide to bind IE promoters (13) and perhaps to interfere with promoter function.

Taken in combination, these results provide strong support for the hypothesis that VP16 activation function stimulates IE gene expression during virus infection, thereby greatly increasing the probability that cells infected with a single HSV particle enter the lytic cycle (2). Why then did Poon and Roizman (26) observe no reduction in viral yields when VP16 was transiently inactivated during the early phase of infection with the R2604 and R2605 ts mutants? Although the explanation is not clear, one possibility is that the VP16 mutations in these isolates

**FIG. 1.** ICP0 and ICP4 transcript levels during infection with V422. Vero and U2OS cells were infected with 10 PFU of V422 or 8MAR per cell in the presence of 200-μg/ml cycloheximide. An 8 mM concentration of HMBA was added to some cultures (+). Aliquots (10 μg) of total cellular RNA were then analyzed for ICP0 and ICP4 transcript levels by primer extension using 5’-32P-labelled 25-mers. Primer extension products were resolved on an 8% sequencing gel. The ICP0 primer (5’-CTAGGGCGGCGTCTGTTGATG-3’) is complementary to residues 71 to 95 of ICP0 mRNA; the ICP4 primer (5’-CGGCGTCTGTTGCGTGG-3’) is complementary to residues 49 to 73 of ICP4 mRNA.

TABLE 2. Fold reduction in IE transcript levels relative to wild-type level

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>V422</th>
<th>1814</th>
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<tbody>
<tr>
<td>ICP0</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>ICP4</td>
<td>65</td>
<td>18</td>
</tr>
</tbody>
</table>

* The levels of ICP4 and ICP0 transcripts produced by V422 and in1814 in the presence of 200-μg/ml cycloheximide were determined by primer extension and were expressed relative to the levels obtained with the corresponding wild-type strains in the same experiment.
partially impair VP16 activation function in vivo at the permissive temperature (33°C), thereby increasing the particle-to-PUF ratio relative to that of wild-type virus. Indeed, these mutations strongly reduced complex formation on TAATGARATTC at 33°C in an in vitro assay (26). If so, then inhibitory effects on entry into the lytic cycle might have been masked at the relatively high multiplicities of infection used (1 PUF/cell, based on titers determined in the absence of HMBA).

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REFERENCES