A Herpes Simplex Virus Type 1 Variant which Fails to Synthesize Immediate Early Polypeptide \( V_{mw} \text{IE63} \)

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SUMMARY
We report the isolation of a variant (X2D) of herpes simplex virus type 1 strain 17 which has a deletion of \( 5 \times 10^6 \) mol. wt. in the long unique and long inverted repeat regions, such that one copy of the immediate early (IE) gene 1 and two unique open reading frames coding for polypeptides of 20K and 22K are deleted. The mutant X2D synthesizes reduced levels of \( V_{mw} \text{IE110} \), and also apparently fails to synthesize \( V_{mw} \text{IE63} \), at both the protein and RNA levels, despite there being no apparent deletion in the coding or controlling regions of the IE2 gene. X2D also fails to synthesize the thymidine kinase polypeptide but exhibits normal growth characteristics in tissue culture.

INTRODUCTION
The genome of herpes simplex virus type 1 (HSV-1) is approximately 150 kb in length or \( 100 \times 10^6 \) mol. wt. and comprises two unique DNA regions (UL and US), each bounded by a set of inverted repeats (IRL/TRL and IRs/TRs) (Fig. 1). Infection with the virus causes the induction of three temporal classes of polypeptides: immediate early (IE), early and late (Honess & Roizman, 1974, 1975; Marsden et al., 1976).

There are five major immediate early genes, IE1, 2, 3, 4, 5 with polypeptide products \( V_{mw} \text{IE110} \), 63, 175, 68, 12 respectively and their genome locations are illustrated in Fig. 1 (Clements et al., 1979). The IE genes 1 and 3 are contained within the repeats and are therefore diploid. The properties and functions of the immediate early polypeptides are not fully understood although studies using temperature-sensitive (ts) mutants and deletion mutants have given a limited amount of information, namely: (i) \( V_{mw} \text{IE175} \) is essential for the expression of early and late polypeptides (Dixon & Schaffer, 1980; Preston, 1981), although one copy is sufficient for normal growth in tissue culture (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987); (ii) one copy of \( V_{mw} \text{IE110} \) is sufficient for lytic growth \textit{in vitro} (Brown et al., 1984; Harland & Brown, 1985) and recent work suggests that this polypeptide may be non-essential (Stow & Stow, 1986); (iii) \( V_{mw} \text{IE12} \) has also been shown to be non-essential for growth in tissue culture (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987); (iv) deletion mutants in \( V_{mw} \text{IE68} \) exhibit host range dependence, growing normally in some cell lines but not in others (Sears et al., 1985; Ackermann et al., 1985); (v) finally, \( ts \) mutants in \( V_{mw} \text{IE63} \) have recently been isolated indicating that this polypeptide may be essential for late gene expression (Sacks et al., 1985).

Using plasmid-based expression systems both \( V_{mw} \text{IE175} \) and \( V_{mw} \text{IE110} \) have been shown to stimulate early and late gene expression (O'Hare & Hayward, 1985). \( V_{mw} \text{IE63} \) on its own has no effect on early and late genes but in conjunction with \( V_{mw} \text{IE175} \) and \( V_{mw} \text{IE110} \) causes increased transcription from a late promoter (Everett, 1986).

Immediate early polypeptide synthesis is stimulated by a virion component (\( V_{mw} \text{IE65} \)) (Batterson & Roizman, 1983; Campbell et al., 1984) interacting with upstream regulatory sequences e.g. TAATGARAT (Preston et al., 1984; Gaffney et al., 1985).

During the isolation of HSV genomes lacking restriction endonuclease cleavage sites (Brown...
et al., 1984; Harland & Brown, 1985; Brown & Harland, 1987), it was found that sequences containing the IR\textsubscript{r}/TR\textsubscript{r} genome regions, including one copy of IE gene 1, could be deleted from both HSV-2 strain HG52 and from an HSV-1 strain 17/HG52 recombinant (R12/5). Until now, despite the analysis of over 5000 plaques of HSV-1 strain 17 (unpublished observation), no genomes containing deletions have been detected.

Deletions have been described in other parts of the HSV genome, notably TR\textsubscript{S} and US (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987). These variants, which are viable in tissue culture, lack the US genes 8, 9, 10, 11, 12 and contain only one copy of OR1\textsubscript{S} (McGeoch et al., 1985; Rixon & McGeoch, 1985; Stow, 1982).

Here we report the isolation of a viable mutant (X2D) of HSV-1 strain 17, which has a deletion of approximately 5 × 10\textsuperscript{6} mol. wt. spanning the U\textsubscript{r}/IR\textsubscript{r} junction from map positions 0-761 to 0-81. The X2D genome structure and polypeptide profile are described. As expected from the location of the deletion, this mutant underproduces V\textsubscript{mw}IE110 but surprisingly fails to express V\textsubscript{mw}IE63 at both the protein and RNA levels.

**METHODS**

**Cells.** BHK C13 cells (Macpherson & Stoker, 1962) were propagated in Eagle's medium containing twice the normal concentration of vitamins and amino acids, 5% (v/v) tryptose phosphate broth and 10% (v/v) calf serum and incubated at 31 °C for 48 h. Viral DNA was isolated by extraction with SDS and phenol and precipitation with ethanol. The DNA was digested with various restriction enzymes using the manufacturer's recommended conditions. Digests were treated with ethidium bromide (0.5 µg/ml) and then precipitated/DMSO boost technique described by Stow & Wilkie (1976). Single plaques obtained from XbaI-digested DNA were isolated, stocks were grown on 50 mm Petri dishes and titrated.

**Preparation of virion DNA.** BHK C13 cell monolayers in 80 oz roller bottles were infected at a multiplicity of 0-003 p.f.u./cell and incubated at 31 °C until the c.p.e. was complete. Infected cells were harvested by a modification of the technique of Lonsdale (1979). Viruses. Virus stocks were grown and titrated in BHK C13 cells as described previously (Brown et al., 1973). The parental strain was HSV-1 Glasgow strain 17 (Brown et al., 1973). Isolation and characterization of the mutant X2 has been previously described (Brown et al., 1984). It lacks two of the XbaI sites contained in the parental strain at map positions 0-07 and 0-63.

**Transfection of virus DNA.** Intact and XbaI-digested HSV DNA (0.2 to 2 µg/plate) was transfected onto semi-confluent monolayers of BHK C13 cells in 50 mm Petri dishes using the calcium phosphate precipitation/DMSO boost technique described by Stow & Wilkie (1976). Single plaques obtained from XbaI-digested DNA were isolated, stocks were grown on 50 mm Petri dishes and titrated.

**Preparation of virion DNA.** BHK C13 cell monolayers in 80 oz roller bottles were infected at a multiplicity of 0-003 p.f.u./cell in the presence of 32P, and incubated at 31 °C until the c.p.e. was complete. Infected cells were harvested by centrifugation and cytoplasmic virus by using treatment with Nonidet P40 (NP40); the DNA was extracted from the cell-released and cytoplasmic virus by treatment with SDS and phenol (Wilkie, 1973; Stow & Wilkie, 1976) and further purified by CsCl gradient centrifugation. A quantitative estimation of the DNA was made by electrophoresis with a known standard in agarose gels containing ethidium bromide (0.5 µg/ml).

**Restriction enzyme analysis of virus genomes.** Restriction enzyme analysis of single plaque isolates was carried out by a modification of the technique of Lonsdale (1979). Five × 10\textsuperscript{6} BHK C13 cells were infected at a m.o.i. of 10 p.f.u./cell in the presence of 32P, in phosphate-free Eagle's medium containing 1% (v/v) calf serum and incubated at 31 °C for 48 h. Viral DNA was isolated by extraction with SDS and phenol and precipitation with ethanol. The DNA was digested with various restriction enzymes using the manufacturer's recommended conditions. Digests were analysed by electrophoresis on agarose gels of appropriate concentrations (0.5% to 1.0%) in TBE buffer (89 mm-Tris-borate, 89 mM-boric acid and 2 mM-EDTA). The gels were air-dried and exposed to Kodak X1 film. When the restriction enzyme profiles indicated mixtures of virus, they were plaque-purified three times before further restriction enzyme analysis.

**Recombinant DNA plasmids.** The genomic locations of plasmids pMC9 and pGX12 (kindly supplied by Dr C. M. Preston and Dr A. J. Davison respectively) are shown in Fig. 4. Plasmid pMC9 extends from the BamH1 site (map positions 0-74 to 0-762) and plasmid pGX12 contains the BamH1 b sequences (map positions 0-75 to 0-805).

**Plasmids KpnI and KpnIb** (spanning map positions 0-285 to 0-322) and KpnIb (spanning map positions 0-945 to 1-000) were also used as controls for dot blot hybridization. The former does not code for immediate early transcripts, while the latter codes for IE3 and IE5 transcripts (Clements et al., 1979). Both these plasmids were kindly supplied by Dr A. J. Davison.

**Preparation of plasmid DNA.** Plasmid DNA was obtained by the method of Birnboim & Doly (1979) and further purified in CsCl/ethidium bromide gradients (Maniatis et al., 1982).

**Southern blotting.** Restriction enzyme-digested DNA was electrophoresed on agarose gels in E buffer (36 mM-Tris–HCl, 30 mM-NaH\textsubscript{2}PO\textsubscript{4}, 2H\textsubscript{2}O and 1 mM-EDTA) containing ethidium bromide (0.5 µg/ml) and then transferred to nitrocellulose sheets (BA85; Schleicher & Schüll) by the method of Southern (1975). These were prehybridized at 75 °C in 6 × SSC (1 × SSC is 15 mm-Trisodium citrate and 150 mm-NaCl), 5 × Denhardt's buffer (1 × Denhardt's is 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone and 0.02% Ficoll), 0.1 mg/ml
**HSV deletion mutants**

salmon sperm DNA and 10 mM-Tris–HCl pH 7.5 and then hybridized to either nick-translated DNA (Rigby et al., 1977) or 32P-labelled RNA (Clements et al., 1977). Following extensive washing at 60 °C in 2 × SSC, 0.1% SDS and 5 mM-Na2HPO4 pH 7, the sheets were air-dried and exposed for autoradiography.

**Dot blots.** Dot blots were carried out by spotting 1 μg of denatured plasmid DNA onto nitrocellulose (BAR85; Schleicher & Schüll). The DNA was denatured by incubation in NaOH at a final concentration of 0.1 M at room temperature for 25 min, neutralized by adding ammonium acetate to a final concentration of 1 M and spotted onto the nitrocellulose, which had been presoaked in 1 M ammonium acetate. After absorption, the nitrocellulose was washed in 6 × SSC and then baked in a vacuum oven for 2 h at 80 °C. Prehybridization and hybridization to labelled RNA (2 × 106 c.p.m.) was carried out as above. After extensive washing the radioactivity bound to the nitrocellulose was counted in a scintillation counter.

**Preparation and analysis of HSV immediate early polypeptides.** The procedure was essentially as described by Preston et al. (1978) as modified by Brown et al. (1984), i.e. the infected cells were harvested in 0.75 ml extraction buffer [100 mM-Tris–HCl pH 8.0, 10% glycerol, 0.5% NP40, 0.5% sodium deoxycholate and 0.2 mM-PMSF (Zweig et al., 1980)]. The infected cell polypeptides were run on either 7.5%, single concentration or 5 to 12.5% gradient SDS–polyacrylamide gels (Marsden et al., 1976, 1978).

**Immunoprecipitation.** The method used was essentially that of Zweig et al. (1980) as modified by Johnson et al. (1986).

**Isolation of labelled HSV immediate early RNA.** Immediate early RNA was prepared by the method described by Clements et al. (1977). At 7 h post-infection cytoplasmic RNA was extracted. The cells were washed twice with phosphate-buffered saline (PBS), scraped into 5 ml PBS and pelleted by centrifugation. The cell pellet was resuspended in 1 ml lysis buffer containing 150 mM-NaCl, 10 mM-Tris–HCl pH 7.8, 1.5 mM-MgCl2 and 0.05% NP40. The nuclei were removed by centrifugation. The supernatant was mixed with 1 ml of a buffer containing 7 m-urea, 350 mM-EDTA, 10 mM-Tris–HCl pH 7.8 and 1% SDS, extracted three times with phenol:chloroform (1:1) and once with chloroform; the RNA was then precipitated with three vol. ethanol at −70 °C (Maniatis et al., 1982). The RNA from 107 BHK cells was resuspended in 1 ml H2O. For each hybridization experiment 2 × 107 c.p.m. was used.

**Virus growth properties.** One-step growth experiments were carried out as described by Brown & Harland (1987). The samples were harvested at 0, 2, 4, 6, 8, 12 and 24 h post-infection. Longer term growth experiments involved infecting cells at a multiplicity of 0.001 p.f.u./cell and harvesting samples at 0, 2, 4, 12, 24, 48 and 72 h. Twenty-four h yield experiments were carried out in both Vero and BHK C13 cells (4 × 106 cells), the progeny virus was titrated on both cell types.

**RESULTS**

**Isolation of an HSV-I genome with a 5 × 106 mol. wt. deletion in IR1/UL.**

During attempts to isolate an HSV-1 strain 17 genome lacking XbaI restriction endonuclease sites, selection enrichment was carried out on the mutant X2 which lacks two of the four wild-type XbaI sites as described previously (Brown et al., 1984). Briefly X2 DNA was cleaved with an excess of XbaI and transfected onto BHK C13 monolayers. Single plaques were isolated and subjected to restriction enzyme analysis. During this analysis a variant was isolated whose profile was markedly different from X2 but did not indicate the removal of an XbaI site (data not shown). The variant (X2D) on BglII digestion (Fig. 2a) showed a missing a band and the f band had an altered migration (Fig. 2b, lane 1). In addition a novel band of 8 × 106 mol. wt. was present below i/j. These changes could be explained by a deletion of around 5 × 106 mol. wt. within IR1/UL, converting BglII f from a 13 × 106 mol. wt. band to an f' band of 8 × 106 mol. wt., running just below BglII i/j. Also the joint fragments containing BglII f (a and c) would be altered such that BglII c (19.5 × 106 mol. wt.) would now be a c' band of 12.5 × 106 mol. wt., running just below the BglII f fragment, and BglII a (24 × 106 mol. wt.) would now run as an a' band of 19 × 106 mol. wt., appearing in the BglII c position.

The X2D genome structure was confirmed by other restriction enzyme cleavage patterns. For the HpaI digest shown in Fig. 3(a) and (b) compare lane 1 (X2D) to lane 2 (X2); two new bands are present in lane 1, one (0.25 M) of 11 × 106 mol. wt. running with b and one (0.5 M) of approximately 1.7 × 106 mol. wt. running below s and HpaI v is absent. The pattern is interpreted as follows: HpaI m has a 1.8 × 106 mol. wt. deletion located within IR1 sequences, therefore generating a novel 0.5 M band of 1.7 × 106 mol. wt. whereas HpaI m generated from TR1 is unaltered and migrates normally. Thus joint fragments containing m, usually 0.5 M, will now consist of undeleted 0.25 M copies, a and d, and deleted 0.25 M copies, a' and d', and a' would run as the novel 0.25 M 11 × 106 mol. wt. band comigrating with b, while the d' band would run
Fig. 1. Structure of the HSV-1 genome showing UL and US flanked by IRL/TRL and IRs/TRs respectively, and the map positions of the five major immediate early polypeptide transcripts (Clements et al., 1979).

Fig. 2. (a) BglII map for the DNA of HSV-1 strain 17. (b) Autoradiographs of BglII restriction digests of HSV-1 DNA 32P-labelled in vivo (0.6% agarose). Lane 1, X2D; lane 2, X2. Missing bands in X2D are marked ▯, and novel bands are designated by the letter of the band from which they were derived plus a prime symbol (').

about 7.2 × 10^6 mol. wt., comigrating with g/h and is therefore not detected. As HpaI v is absent and HpaI m is deleted, the intervening fragment, HpaI r, would be expected to be absent; indeed the q/r band in X2D is reduced in intensity relative both to the parental X2 q/r band and to the equivalent 2 μ o/p band in X2D. A longer exposure of the lower part of the HSV-1 17 lane is shown to illustrate the presence of v which is faint in the complete lane as this is less exposed than the X2D lane. The data are therefore consistent with a deletion of approximately 4.9 × 10^6 mol. wt. at the UL/IRL junction.

Southern blotting analysis confirmed that the deletion was in the order of 5 × 10^6 mol. wt. A BamHI b probe (position illustrated in Fig. 4) hybridized to a b' band of 1.7 × 10^6 mol. wt., compared to the wild-type b band at 6.7 × 10^6 mol. wt. (results not shown). To map the deletion within UL more precisely, a probe, pMC9, corresponding to the unique portion of BamHI b (Fig. 4b) was hybridized to X2 (Fig. 5, lane 1) and X2D (lane 2) DNA cleaved with BamHI/SmaI/HpaI. The SmaI fragments from HpaI s (Fig. 5) are all present (Perry, 1986), indicating that the deletion does not extend past the HpaI s/v junction but HpaI v (1240 bp) is
Fig. 3. (a) *HpaI* map for the DNA of HSV-1 strain 17. (b) Autoradiographs of *HpaI* restriction digests of HSV-1 DNA 32P-labelled in vivo (0.8% agarose). Lane 1, X2D; lane 2, X2 and lane 2 (i) is a longer exposure of the lower part of lane 2 to show the presence of *HpaI* v clearly. Reduced or missing bands in X2D are marked ▶ and novel bands are designated by the letter of the band from which they were derived plus a prime symbol (').
Fig. 5. Autoradiograph of a Southern blot in which nick-translated pMC9 (see Fig. 4b) was hybridized to X2 (lane 1) and X2D (lane 2) DNA which had been digested with BamHI/HpaI/SmaI. For the location of the bands in the genome see Fig. 4(c). The missing band in X2D is marked ▲. Sizes of the bands are in base pairs on the left hand side.

Fig. 6. (a) Immediate early protein extracts (see Methods) of X2-infected (lane 1), X2D-infected (lane 2) and mock-infected (lane 3) HFL cells run on a 5 to 12.5% gradient polyacrylamide gel. Molecular weights (× 10⁻³) are given on the left-hand side. (b) Immunoprecipitation using an antiserum raised against the carboxy terminus peptide of VₘₜₐIE110. Lanes 1 and 4, mock-infected; lanes 2 and 5, X2D-infected and lanes 3 and 6, X2-infected cell extracts. The immunoprecipitation was carried out in the presence (lanes 1, 2 and 3) or absence (lanes 4, 5 and 6) of the relevant peptide. The specificities of the precipitation of VₘₜₐIE110 and the lower mol. wt. bands around actin (A) is demonstrated by inhibition by the peptide. (c) Immediate early protein extracts (see Methods) of X2D-infected (lane 1), X2-infected (lane 2 and 4), 17-infected (lane 3) and mock-infected (lane 5) HFL cells run on a 7.5% polyacrylamide gel. Molecular weights (× 10⁻³) are given on the left hand side.

absent from X2D. As fragments of up to 200 bp will run off the gel, the deletion extends at least as far as 200 bp to the right of the HpaI s/v junction (Fig. 4).

Effect of the deletion on immediate early polypeptide synthesis

The deletion in X2D removes the 3' portion of IE gene 1 located in IRL and terminates at least 500 bp downstream from the mRNA 3' end of IE gene 2 (Fig. 4a and c).

Immediate early polypeptides were prepared and analysed by SDS–PAGE. VₘₜₐIE110 was detected in cells infected with X2D (Fig. 6a, lane 2) and a reduced level was seen in immunoprecipitation experiments using an antiserum raised against the carboxy terminus of VₘₜₐIE110 (Perry et al., 1986). The antiserum specifically precipitated a polypeptide of apparent mol. wt. 110 × 10³ and several bands of lower mol. wt. (Fig. 6b, lanes 5, 6), which were not precipitated in the presence of the relevant peptide (Fig. 6b, lanes 2, 3). A reduction in the level of VₘₜₐIE110 precipitated from X2D (Fig. 6b, lane 5) extracts as compared to control X2 (Fig. 6b, lane 6) extracts was consistently found in several experiments.

A striking feature of the immediate early polypeptide profile of X2D was the apparent lack of VₘₜₐIE63 (Fig. 6a, compare lanes 1 and 2). Due to the large number of host proteins and the poor
HSV deletion mutants

resolution in this region on gradient gels, the infected cell polypeptides were analysed on 7.5% single concentration gels (Fig. 6c). The results clearly suggest that VmwlE63 is either absent or is present in greatly reduced amounts. The absence of IE68 on these gels is due to a number of factors: it is not a major immediate early polypeptide; it is poorly labelled with [35S]methionine and under long labelling conditions it is unstable. Also it should be noted that IE110 comigrated with a host polypeptide in this single concentration gel which uses the same extract as in the gradient gel illustrated. Fig. 6 shows the results of infection of HFL cells, but similar results were obtained using BHK C13 cells (results not shown).

The levels of virus-specific RNAs present in X2D-infected cells were examined. Radiolabelled, cytoplasmic, immediate early RNA was hybridized to Southern blots of HpaI-digested HSV DNA. In the strain 17 control (Fig. 7, lane 2) IE1 RNA hybridized to HpaI m, a and d, IE3 RNA hybridized to HpaI c, g, a and d, IE4 RNA hybridized to HpaI c and a, IE5 RNA hybridized to HpaI g and d bands, and IE2 RNA hybridized to HpaI s (Clements et al., 1979 and Fig. 1 and 3a). X2 immediate early RNA produced an identical hybridization pattern (not shown). In contrast X2D RNA (lane 3) failed to hybridize to HpaI s. Thus IE2 mRNA specified by X2D cannot be detected. Also, less X2D RNA hybridized to HpaI m than in strain 17, indicating a reduced amount of IE1 RNA. Despite the slightly lower levels of hybridization to the X2D lane, we would still expect to see hybridization to HpaI s, if IE mRNA levels comparable to the wild-type were present.

To determine the sensitivity of IE2 RNA detection, a dot blot assay was carried out. Labelled IE RNA (2 × 10⁶ c.p.m.) from X2D, strain 17 and mock-infected cells was hybridized to: (i) pMC9 to detect the level of IE2 RNA present; (ii) plasmid KpnIk containing IE3 and IE5 genes, to determine whether X2D and strain 17 infected cells made the same amount of their immediate early RNA; (iii) plasmid KpnIm, containing no immediate early genes, as a background (see Methods for description of plasmid map locations). Different dilutions of strain 17 and X2D RNA with the input counts being equalized using mock-infected cell RNA, were used to quantify the assay sensitivity. The radioactivity (Table 1) above mock levels indicates

Fig. 7. Autoradiograph of Southern blots of HpaI-digested strain 17 DNA (0.8% agarose), to which HSV-1 strain 17 IE RNA 32P-labelled in vivo (lane 2), X2D IE RNA (lane 3) or nick-translation strain 17 DNA (lane 1) was hybridized. The band absent in X2D is marked ◆, and that reduced in intensity ▲.
Table 1. **Sensitivity of detection of IE2 RNA**

<table>
<thead>
<tr>
<th>Dilution (fold)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
</tr>
</thead>
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<tr>
<td>Plasmid used (1 μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMC9 (IE2)</td>
<td>41*</td>
<td>37</td>
<td>30</td>
<td>34</td>
<td>32</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Kpnlk (IE3 + 1R5)</td>
<td>36</td>
<td>309</td>
<td>320</td>
<td>341</td>
<td>230</td>
<td>190</td>
<td>109</td>
</tr>
<tr>
<td>Kpnlm (no IE)</td>
<td>26</td>
<td>39</td>
<td>25</td>
<td>31</td>
<td>38</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

* C.p.m. bound to filter. Input virus (or mock-infected cell) RNA was 2 x 10⁶ c.p.m.

Table 2. **Twenty-four h yields in BHK and Vero cells**

<table>
<thead>
<tr>
<th>Virus grown in</th>
<th>Vero</th>
<th>BHK</th>
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<tr>
<td></td>
<td>Titrated in Vero</td>
<td>Titrated in BHK</td>
</tr>
<tr>
<td>HSV-1 strain 17</td>
<td>1.8*</td>
<td>2.0</td>
</tr>
<tr>
<td>X2</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>X2D</td>
<td>1.9</td>
<td>1.7</td>
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</tbody>
</table>

* Yield given in p.f.u. x 10⁸/4 x 10⁶ cells.

The level of RNA specific to the cloned fragments. Table 1 indicates that X2D and 17 specify equal levels of IE3 RNA and IE5 RNA, and the sensitivity is such that X2D specifies no more IE2 RNA than mock-infected cells. At all dilutions down to and including 1/16, strain 17 contained detectable levels of IE2 RNA. However, at a 1/32 dilution, strain 17 showed hybridization levels comparable to those of X2D and mock-infected cell RNA. The conclusion of this experiment is that X2D extracts contain at most 1/16 (6.7%) of the wild-type level of IE2 RNA.

The general cell polypeptide profile of X2D was indistinguishable from that of strain 17 and X2 with the exception that a 43K polypeptide believed to be the thymidine kinase was absent from X2D. Confirmation that X2D was thymidine kinase-negative was obtained using bromodeoxycytidine (results not shown) (Stow et al., 1978).

**Growth characteristics of X2D**

A one-step growth curve over a 24 h period in BHK C13 cells showed no marked difference in growth properties between X2D, X2 and strain 17. The lag period of X2D appeared slightly longer than that for X2 and 17 (Fig. 8a), but as this is a very variable property, the significance is uncertain. X2D, X2 and strain 17 also showed almost identical growth characteristics over several rounds of replication (Fig. 8b).

Sacks et al. (1985) have identified ts mutants in VmwlE63, suggesting that this protein is essential for growth in tissue culture. These authors used Vero cells, whereas we used BHK or HFL cells. We also carried out 24 h yield experiments at 5 p.f.u./cell in both Vero and BHK C13 cells with strain 17, X2 and X2D, and titrated the progeny virus on both cell types. Table 2 shows that the yields of the three viruses obtained from the two cell lines were very similar and the titres measured on the two cell lines were identical.

**DISCUSSION**

In this paper we report the isolation and characterization of an HSV-1 strain 17 mutant with a 5 x 10⁶ mol. wt. deletion within IR₁ and U₁. The deletion removes the 3' end of the IR₁ copy of IE1 and two unique open reading frames coding for polypeptides of 20K and 22K for which transcripts have been identified (Perry, 1986). The deletion terminates at least 500 bp downstream from the 3' end of the IE2 transcript (Whitton et al., 1983) (Fig. 4a). As McLauchlan et al. (1985) have shown that almost all of the sequences required for efficient termination of HSV genes are situated within 50 to 150 bp of the polyadenylation signal, it is
unlikely that the deletion has a cis-acting effect on IE2 transcription. Thus the lack of V\textsubscript{mw63} is probably due to a secondary defect in either (i) essential 5' or 3' regulatory regions such as TAATGARAT (Gaffney et al., 1985), the TATA box or RNA transcription start site or (ii) within the protein coding sequences. Alternatively there could be a mutation in a protein that transactivates IE2. This mutated protein could either be unrelated to the deletion or alternatively could be one of the two open reading frames removed by the deletion, one of which, the 20K polypeptide, is conserved in varicella-zoster virus (Davison & Scott, 1986 and personal communication). As regards this latter possibility it is worth mentioning that the deletions in IR\textsubscript{1} of HSV-2 strain HG52 reported by Harland & Brown (1985) did not extend into the region of the two predicted open reading frames in HSV-1 and had no effect on V\textsubscript{mw63} expression.

Given that Sacks et al. (1985) have identified four ts mutations which map within IE2 in HSV-1 strain KOS, the isolation of a mutant that grows normally in tissue culture and yet produces no, or at most 10% of normal levels of V\textsubscript{mw63} is somewhat puzzling. A number of explanations could be considered. (i) X2D could be a host range mutant similar to the V\textsubscript{mw68} mutants described by Sears et al. (1985) and Ackermann et al. (1985). As it grows equally well in Vero cells [the cells used by Sacks et al. (1985)] and BHK C13 cells, the possibility is unlikely; (ii) in cells infected with strain 17, another immediate early polypeptide can substitute for V\textsubscript{mw63}, whereas in cells infected with strain KOS this is not possible; (iii) a non-functional ts V\textsubscript{mw63} may hinder protein–protein or protein–DNA interaction rendering a virus non-viable, whereas the complete lack of production of V\textsubscript{mw63} would not do this and may therefore result in a viable virus; (iv) lastly, X2D produces a very low amount of V\textsubscript{mw63} which is adequate for
normal lytic growth. It will be interesting to see if X2D will replicate in vivo as well as wild-type virus. To answer the question of whether a low level of V<sub>mwl</sub>IE63 is present we are currently raising antisera against the carboxy and amino termini of V<sub>mwl</sub>IE63.

X2D also fails to express the thymidine kinase polypeptide. Given the relatively common occurrence of thymidine kinase-negative virus, this is most likely to be the result of an unrelated secondary mutation. However, a connection between the apparent absence of V<sub>mwl</sub>IE63 and the thymidine kinase polypeptide cannot be ruled out.

The origin of the X2D virus with the deletion is open to speculation. The most likely explanation is that it arose by a spontaneous deletion, although it is possible that it could have arisen either by a mutagenic effect of the XbaI treatment or the transfection process. The X2D variant contains the first detected deletion in an HSV-1 strain 17 genome, despite analysis of more than 5000 plaques. Therefore HSV-1 strain 17 is considerably more stable than HSV-2 strain HG52, where deletions in TR<sub>L</sub> and IR<sub>L</sub> arose in the elite stock at a frequency of 24% (Harland & Brown, 1985). Deletions also occurred in other wild-type strains of HSV-1 and HSV-2 at lower frequencies (Harland & Brown, 1985); and in the 17/HG52 recombinant R12-5, deletions in both the 17 and HG52 copies of the long repeat have been reported (Brown et al., 1984). Thus deletions around the TR<sub>L</sub>/IR<sub>L</sub> repeats occur at different frequencies in different strains, possibly due to more mutagenic recombination/replication enzymes in certain strains or to larger or more frequent sequence reiterations in these regions. Studies on deletions and illegitimate recombination in viruses, between viruses and chromosomes and in prokaryotic systems indicate a role for short G + C rich direct repeats at the regions of strand exchange or endpoints of deletions (Stringer, 1982; Ruley & Fried, 1983; Bullock et al., 1984).

Sequence analysis of HSV-1 has shown that there are three groups of tandemly reiterated sequences of high G + C content within the TR<sub>L</sub>/TR<sub>S</sub> repeat region of the genome (Rixon et al., 1984) which may produce regions of instability. Other regions of the genome in which deletions have been reported include TR<sub>S</sub> and the adjacent unique sequences (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987). In one case at least, the deletion has been shown to occur between short direct repeats (Umene, 1986).

The loss of one copy of the long repeat has been shown to have little effect on virus growth properties in vitro (Brown et al., 1984; Harland & Brown, 1985), although the effect on neurovirulence and latency is still under study.

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