A herpes simplex virus type 1 recombinant with both copies of the Vmw175 coding sequences replaced by the homologous varicella-zoster virus open reading frame

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Varicella-zoster virus (VZV) gene 62 encodes a protein with a predicted M, of 140000 (VZV 140K) that shares considerable amino acid homology with the immediate early (IE) regulatory protein Vmw175 of herpes simplex virus type 1 (HSV-1) and is believed to be its functional equivalent. We have tested this hypothesis by insertion of VZV gene 62 (expressed from the HSV-1 IE3 promoter) into both IE3 gene loci in the short region repeats of the HSV-1 genome. The parent virus used for this manipulation was D30EBA, which is a variant of HSV-1 from which the majority of the Vmw175 coding sequences have been deleted. Like other HSV-1 viruses lacking Vmw175 function, D30EBA is able to grow only in cell lines which express Vmw175 constitutively. The resulting recombinant virus, HSV-140, is able to propagate (but unable to form obvious plaques) on normal cell lines. The properties of HSV-140 were studied by monitoring the time course of polypeptide expression and DNA replication during normal infection. We found that at high multiplicity HSV-140 synthesized apparently normal amounts of many viral polypeptides but that the expression of certain late genes was reduced; this slight defect may be related to less efficient DNA replication by HSV-140. At low multiplicity HSV-140 expressed viral proteins inefficiently. Surprisingly, VZV 140K was produced in large amounts at later times of a normal infection, indicating that the polypeptide fails to autoregulate the IE3 promoter. The results strongly suggest that VZV 140K is able to perform most of the functions of Vmw175 during growth of HSV-1, but that differences in detail lead to less efficient virus growth.

Introduction

Varicella-zoster virus (VZV) is a common human pathogen which causes chickenpox on initial exposure and shingles on subsequent reactivation from latency. It is a member of the neurotropic alphaherpesvirinae subfamily and is genetically closely related to herpes simplex virus type 1 (HSV-1) (Davison & Scott, 1986; McGeoch et al., 1988). At present, in contrast to the increasingly sophisticated knowledge of the molecular biology of HSV, studies on the mechanisms of gene regulation during VZV infection are at an early stage. This is principally a result of the growth properties of VZV in tissue culture, which make the isolation of single plaques and establishment of high titre stocks difficult. However, the availability of the complete DNA sequence of VZV (Davison & Scott, 1986) and the use of transfection and expression systems allow individual genes, selected from the 71 predicted major open reading frames (ORFs), to be studied. This paper concerns the product of VZV gene 62, whose predicted product (VZV 140K) is closely related to the regulatory polypeptide Vmw175 (or ICP4) of HSV-1 (McGeoch et al., 1986).

Gene regulation during HSV-1 infection in tissue culture has been extensively studied (for reviews see Wagner, 1985; Everett, 1987a). The viral genes are expressed in three broad classes termed immediate early (IE), early and late. The IE genes are transcribed by the unmodified host RNA polymerase II at the onset of infection (Costanzo et al., 1977). Of the five IE genes, at least four can function as transcriptional activators of viral (and in some cases cellular) gene expression. The product of IE gene 3, Vmw175, is absolutely required for the expression of early and late genes and is also required for the repression or autoregulation of IE gene expression (Preston, 1979; Watson & Clements, 1980). HSV-1 viruses with temperature-sensitive (ts) lesions in Vmw175 are unable to grow at the non-permissive temperature.

Homologues of Vmw175 have been identified from DNA sequence information in all the other alphaherpesviruses so far analysed, including VZV (Davison &...
The product of VZV genes 62 and 71 (the identical gene is present in both copies of the short region repeats; Fig. 1b) has a predicted Mr of 140K and for several reasons is believed to be the VZV equivalent of Vmw175. The genes are located in equivalent positions and there is extensive predicted amino acid sequence conservation between them (McGeoch et al., 1986). Also, VZV 140K has been shown to be a potent activator of gene expression in transient transfection assays (Everett & Dunlop, 1984; Everett, 1984; Inchauspe et al., 1989) and the regions of the Vmw175 polypeptide that have been shown to be most important for its transactivation and repression phenotypes are the most highly conserved in 140K (Paterson & Everett, 1988a; DeLuca & Schaffer, 1988). These same regions are also highly conserved in the other alphaherpesvirus homologues of Vmw175 (Grundy et al., 1989; Cheung, 1989; Vleck et al., 1989). Four IE proteins have been identified in VZV-infected cells using cycloheximide reversal experiments, the largest of which has an apparent molecular weight of 185000 and has been proposed to be the product of gene 62 (Shiraki & Hyman, 1987). Perhaps the most compelling evidence that VZV 140K and HSV-1 Vmw175 are functionally homologous is that HSV-1 mutants with lesions in Vmw175 can be complemented by either transfected plasmids or transformed cell lines which express VZV 140K (Felser et al., 1987, 1988).

In this paper we have extended such studies by construction of a virus (HSV-140) which contains VZV 140K in place of the coding sequences for Vmw175 in the HSV-1 genome. HSV-140 was constructed from D30EBA (Paterson & Everett, 1990), an HSV-1 derivative that has the majority of the Vmw175 coding sequences deleted, and a plasmid in which 140K is expressed from the IE3 transcription regulatory signals. Unlike D30EBA, HSV-140 is able to grow in normal tissue culture cells. We have further investigated the properties of VZV 140K and its complementation of Vmw175 by studies on the growth, polypeptide expression and DNA replication of HSV-140. We found that although VZV 140K is able to complement for the absence of Vmw175, HSV-140 is partially defective in comparison to HSV-1.

**Methods**

**Plasmids.** Plasmid pGD140, which contains the VZV 140K coding sequences in an HSV-1 IE3 transcription unit (Fig. 1a) was constructed from fragments derived from pGX161, pJ38 and p140BTE. Plasmid pGX161 (kindly provided by Dr C. M. Preston) has been described previously (Preston & Fisher, 1984) and contains the promoter and upstream regulatory sequences of the HSV-1 IE3 gene (nucleotides −331 to +27) between the HindIII and BamHI sites of pAT153.

Plasmid pJ38 (kindly provided by Dr T. Paterson) is a derivative of p175 (Perry et al., 1986) created by insertion of a 12 bp EcoRI linker oligonucleotide into a Nael site within the Vmw175 coding sequences, resulting in an in-frame insertion of four amino acids into the proline codon at position 1236 (Paterson & Everett, 1988a). Plasmid p140 contains VZV gene 62 in a fragment from the Clal site at position −1146 to the terminal PstI site of pVZVSstf (Everett, 1984) inserted between the AccI and PstI sites of a plC9 plasmid from which the EcoRI site had been removed by cutting and filling in (McKee et al., 1986). Plasmid p140BTE is a derivative of p140 which has a unique BgIII site at position +57 (relative to the transcription start site at +1) and a unique EcoRI site four nucleotides beyond the 3′ end of the ORF. Plasmid p140 was subjected to site-directed mutagenesis to create a BgIII site by insertion of an eight nucleotide BglII linker to create p140BT. Plasmid p140BT was linearized by digestion with T4kI, which cuts two nucleotides beyond the end of the gene 62 reading frame, and EcoRI linkers were inserted after filling in the ends to yield p140BTE. Plasmid pGD140 was constructed in a tripartite ligation using the BglII–EcoRI fragment from p140BTE containing the VZV 140K coding sequences, the smaller PstI–BamHI fragment of pGX161 containing the IE3 promoter (and part of the vector sequences) and the smaller PstI–EcoRI fragment of pJ38 which provides the rest of the vector sequences, the C-terminal 62 codons of Vmw175, the 3′ end of the IE3 transcription unit and subsequent HSV-1 DNA through to the SalI site in TR, (Fig. 1a). The C-terminal portion of Vmw175 in this construct follows the TGA stop codon of VZV gene 62. Plasmid p11 (Everett, 1987b) was derived from p110del7 (Perry et al., 1986) by filling in the EcoRI site in the polylinker sequences.

**Cells.** Baby hamster kidney cells (BHK-21 clone 13; Macpherson & Stoker, 1962), were grown in Glasgow Modified Eagle’s medium (GMEM, Gibco) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 10% tryptose phosphate and 10% newborn calf serum. Human foetal lung (HFL) cells were grown in GMEM supplemented with 10% foetal calf serum, penicillin and streptomycin as above.

**Viruses.** Throughout this paper wild-type (wt) virus refers to HSV-1 Glasgow strain 17 syn* (Brown et al., 1973). The HSV-1 derivative D30EBA, which lacks codons 84 to 1270 of the Vmw175 coding sequence (Paterson & Everett, 1990), was used as the parent virus for the construction of HSV-140. D30EBA was propagated on M64A cells, a transformed cell line that expresses Vmw175 and which is equivalent to M65 cells as described by Davidson & Stow (1985).

**Construction of HSV-140.** A mixture containing approximately 1 μg of plasmid pGD140 linearized with PstI, 3 μg of D30EBA viral DNA and 5 μg of calf thymus DNA (as a carrier) were transfected into 2 × 10^6 BHK cells by the method of Stow & Wilkie (1976). Cells were also transfected with viral or plasmid DNA alone as a control. Following transfection, cells were incubated at 37 °C and when c.p.e. was apparent (after 3 to 4 days), the cells and medium from each plate were harvested, sonicated and stored as a virus stock. The presence of the desired recombinant virus was detected by slot blot analysis of total cellular DNA prepared from BHK cells infected with the initial virus stocks. The structure of the recombinant virus genome was analysed by Southern blotting. Finally, HSV-140 was purified from these initial stocks. The structure of the recombinant virus genome was analysed by Southern blotting. Finally, HSV-140 was purified from these initial isolates by limiting dilution.

**Preparation and analysis of viral DNA.** Viral DNA was prepared as total cellular DNA from BHK cell monolayers infected with HSV-140 or wt virus (as detailed in the text) by the method of Stow et al. (1983) and analysed after digestion with BamHI by Southern blotting (Southern, 1975) using nick-translated p140 as a probe (Rigby et al., 1977).
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Labelling and analysis of viral polypeptides. Examination of polypeptide synthesis throughout the growth cycle was performed at 37°C using a 2 h labelling period. Single wells of 24-well Linbro blocks were infected at various multiplicities (as described in the text) and at various times thereafter, washed with phosphate-buffered saline (PBS) and incubated with 15 μCi [35S]methionine (Amersham, >800 Ci/mmol) in 200 μl of PBS. After labelling, the cells were washed and harvested and the labelled proteins analysed on discontinuous 7.5% SDS-polyacrylamide gels as described by Marsden et al. (1978).

Assay of DNA replication. BHK cells in Linbro wells were infected with HSV-140 or wt virus and total cellular DNA was prepared at various times post-infection (as described in the text). DNA samples were treated with RNase (100 μg/ml, 65°C for 1 h) prior to application to a nitrocellulose membrane using a slot blot apparatus (Schleicher and Schuell, Minifold II). Blots were probed with nick-translated p111 (Rigby et al., 1977).

Results

Construction of a virus expressing VZV 140K in place of Vmw175

Virus D30EBA lacks the majority of the Vmw175 coding sequences and is unable to grow in normal cell lines (Paterson & Everett, 1990). Since VZV and plasmids expressing the VZV 140K polypeptide complement HSV-1 viruses with ts mutations in Vmw175 (Felser et al., 1987, 1988), we expected to be able to rescue D30EBA by the introduction of gene 62 of VZV. Hence plasmid pGD140, which contains the VZV gene 62 coding region inserted into an IE3 transcription unit (Fig. 1 a), was constructed. The IE3 sequences 5' and 3' of the VZV 140K coding region allow recombinational insertion into the normal location of IE3 in the short region repeats.

D30EBA viral DNA was cotransfected into BHK cells with linearized pGD140. We expected that any recombinants that contained ORF62 would be able to grow, whereas input viral DNA alone would not lead to virus production. After 4 days c.p.e. was observed in cells cotransfected with viral and plasmid DNA, whereas no c.p.e. was observed in the control transfection with viral DNA alone. Cells and medium combined from both the control and cotransfected plates were sonicated and retained as virus stocks. These stocks were used to infect further BHK cells and total infected cell DNA was prepared. This was initially analysed by DNA slot blots using nick-translated p140 (McKee et al., 1990) as a probe, which showed that only DNA prepared from the cotransfected cells hybridized to VZV sequences (results not shown).

The recombinant viral DNA was further analysed by Southern blotting (Fig. 3). The controls show that neither mock- nor wt HSV-1-infected cell DNA samples hybridize to the p140 probe. The BamHI restriction map for the expected recombinant virus (Fig. 2) predicts that the probe will hybridize to the following fragments on the blot: BamHI [k'] (6.0 kb) and BamHI [y' + n] (7.1 kb) derived from IRs; BamHI [x + y'] (4.1 kb); BamHI [q'] (3.0 kb) derived from TRs. Fragments corresponding in size to the three larger predicted BamHI restriction fragments are clearly observed on the blot. However, the
Fig. 2. The predicted BamHI restriction maps for the short regions of the wt HSV-1 and HSV-140 genomes. VZV gene 62 coding sequences replace both copies of Vmw175 in the short region repeats of the HSV-1 genome in HSV-140; VZV sequences are represented by the stippled box and the limits of the ORFs shown by arrows. The Vmw175 coding sequences in HSV-1 are contained within BamHI q and BamHI y; their replacement by the corresponding gene 62 sequences in HSV-140 results in restriction fragments designated BamHI q' and BamHI y'. BamHI k' represents (q' + s) derived from the IRq/IRn junction. The BamHI site at the cap site of the IE3 gene (present at map units 0.865 and 0.965 approximately in HSV-1) was destroyed during the construction of pGD140 (marked * in HSV-140, see Fig. 1). Therefore BamHI digestion of HSV-140 DNA produces fragments (y' + n) and (x + y'). The sizes (kb) of these four novel fragments containing portions of the VZV gene 62 sequence are shown.

BamHI [q'] fragment, which represents sequences from the genome terminus, was not observed in this experiment. A weakly hybridizing fragment of 3 kb was once observed during the isolation and purification of HSV-140, which probably indicates that viral DNA prepared from infected cells is highly concatemerized with consequent under-representation of the terminal fragment. The simplest interpretation of these results is that an intact copy of VZV gene 62 is present in both IE3 gene loci in the HSV-1 genome in our primary isolate of the recombinant virus HSV-140.

The initial isolate was amplified and plaque-purification under agar was attempted, but despite several attempts no plaques were observed (see below). Virus HSV-140 was subsequently purified by limiting dilution in Linbro wells, such that c.p.e. was observed in 30% or less of wells. Medium was retained from wells that displayed c.p.e. and used to infect cells for total cellular DNA preparation. Southern blot analysis of the resulting DNAs showed all eight stocks of virus to be of the predicted structure with no evidence of contamination. Recombinant viruses carrying only one copy of gene 62 were not detected at any stage, suggesting that HSV-140, with two copies of gene 62, has a strong growth advantage. One of the small scale stocks was selected for a large scale virus preparation which was again checked by Southern blotting. The isolation of HSV-140 on normal BHK cells shows that VZV-140K can functionally replace Vmw175.

Growth properties of HSV-140

HSV-140 was compared to wt virus in a standard plaque assay in BHK cells to assess the extent to which VZV-140K can complement for loss of Vmw175 function. Cells were infected with increasing dilutions of each virus and fixed and stained after 2 days. At lower dilutions both viruses produced extensive c.p.e. although the appearance of c.p.e. during HSV-140 infections was delayed approximately 12 h compared to that during wt virus infections. At increasing dilutions single plaques were obtained with wt virus as normal, but no clear plaques were produced by HSV-140. The assay was repeated several times with independent stocks of HSV-140 and in no case were distinct plaques formed. Similar results were obtained using HFL cells. The results suggest that although HSV-140 is able to grow in the
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Fig. 3. Southern blot analysis of DNA prepared from HSV-140-infected cells. DNA was prepared from infected cells when extensive c.p.e. was apparent. The DNA was digested with *BamHI* and subjected to Southern blot analysis using a nick-translated plasmid p140 probe which contains VZV gene 62 but no HSV-1 sequences. Lane 1, mock-infected cell DNA; lanes 2 and 3, HSV-140-infected cell DNA using two different virus stocks; lane 4, HSV-1-infected (strain 17) cell DNA. The positions of *HindIII* size markers (kb) are shown on the left. The identities of fragments hybridizing to the probe and their predicted sizes (kb) are shown on the right. They were assigned by comparison of their estimated sizes to those predicted from the maps in Fig. 2. The larger fragments in lane 2 represent partial digestion products.

The absence of Vmw175 expression, plaque formation is impaired, at least in the cell types used in these studies.

The numbers of virus particles in the large scale stocks of cell-associated and supernatant HSV-140 virus were determined. HSV-140 cell-associated stocks were routinely around $3 \times 10^{10}$ particles/ml, which was equivalent to the corresponding particle counts of stocks of wt virus produced in parallel. The supernatant virus particle count of HSV-140 was reduced by a factor of 10 compared to the cell-associated virus and the wt supernatant virus stocks. If this reflects a slower rate of virus assembly or release then it may in part explain the lack of plaque formation by HSV-140.

**The time course of HSV-140 viral polypeptide synthesis**

As titres could not be determined by plaque assay for HSV-140 virus stocks, viral polypeptide synthesis in HSV-140- and wt virus-infected cells were compared using 10 p.f.u./cell of wt virus and increasing dilutions of HSV-140. Cells were labelled with $[^{35}S]$methionine from 3 to 6 h post-adsorption (p.a.) and proteins analysed by SDS-PAGE. Infection with dilutions of HSV-140 corresponding to multiplicities of 2500, 500 and 100 particles per cell (p.p.c.) resulted in levels of viral gene expression that were equivalent to the wt virus infection (results not shown). Since an amount of VZV inoculum corresponding to 100 p.p.c. gave viral polypeptide expression similar to 10 p.f.u. wt of virus/cell in this single experiment, this amount of HSV-140 was used in a subsequent time course experiment.

Cells were mock-infected or infected with wt and HSV-140 viruses and labelled between 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 16 to 18 and 22 to 24 p.a. These results are shown in Fig. 4. The protein expression profiles of the two viruses were similar, but a significant difference was the kinetics of expression of a high *M* protein in HSV-140-infected cells. On the basis of its mobility, expression by HSV-140 and Western blotting using a polyclonal rabbit antiserum directed against a fusion protein including part of VZV 140K (data not shown; D. Stephenson & A. Davison, personal communication), the most likely interpretation is that this high *M* band is the VZV 140K polypeptide. It is notable that the kinetics of expression of VZV 140K in HSV-140-infected cells is very different compared to Vmw175 in wt virus-infected cells. Strikingly, VZV 140K was expressed as an early protein and was abundant late in infection. Thus VZV 140K is apparently not subject to autoregulation in HSV-140, which suggests that the
normal repression of IE gene expression may be defective in this virus. It is not possible to determine from these data whether Vmw110, Vmw68 or Vmw63 are also overexpressed during HSV-140 infection.

Most other clearly visible viral proteins are expressed in HSV-140- and wt virus-infected cells in similar amounts and with similar kinetics. However, the expression of certain late gene products (for example, Vmw65 and the major capsid protein) appears to be lower in HSV-140-infected cells. This may be the result of less efficient replication of viral DNA during HSV-140 infection (see below).

Comparison of viral DNA replication during strain 17 and HSV-140 infections

BHK cells were infected with HSV-140 and wt virus and total cellular DNA was prepared at 4, 6, 8, 10 and 16 h p.a. The DNA samples were treated with RNase and analysed in a slot blot experiment to compare the amounts of viral DNA present in the two infections. Nick-translated p111 (which contains HSV-1 IE gene 1 sequences; Everett, 1987b) was used as a probe as it contains sequences common to both viruses. In cells infected with wt virus, DNA accumulation began prior to the earliest time point of 4 h p.a. and continued through to 16 h p.a. (Fig. 5). The onset of HSV-140 DNA replication was delayed 2 to 4 h relative to that of wt virus and the amounts of viral DNA produced were generally reduced; no accumulation of DNA was seen at 4 h p.a. and only very low levels were seen at 6 h p.a. (Fig. 5).

Viral gene expression during infection with HSV-140 is multiplicity-dependent

Several mutants of HSV-1 containing mutations in regulatory functions have been shown to have defects in viral gene expression at low multiplicities in certain cell types (Stow & Stow, 1986; Sacks & Schaffer, 1987; Ace et al., 1989; Everett, 1989). We decided to investigate the
synthesis of viral polypeptides during high and low multiplicity infections of BHK and HFL cells by HSV-140. In an attempt to minimize experimental variation, the virus stocks used in this study were all prepared in parallel from the same batch of cells. BHK or HFL cells were infected at 50, 30, 10 and 3 p.p.c. with HSV-140 or wt virus and labelled with $[^{35}S]$methionine at 10 to 12 h p.a. (Fig. 6). In agreement with the time-course experiment (Fig. 4) at higher multiplicities both viruses expressed similar levels of most viral polypeptides, although the expression of certain late proteins (for example Vmw65 and the major capsid protein) was again reduced in HSV-140 infections. At the lower multiplicities of 10 and 3 p.p.c., HSV-140 expressed reduced amounts of all viral polypeptides compared to wt virus in both BHK and HFL cells. This finding was consistent with several different stocks of HSV-140. Therefore HSV-140 exhibits a multiplicity-dependent gene expression defect which, in the cases examined, is not cell type-dependent.

In addition, the results in Fig. 6 suggest that the ability of HSV-140 to shut off host protein synthesis is significantly impaired. These results cannot be considered conclusive because labelling at late times means that secondary infection by the faster growing wt virus could contribute to the observed shut off of host protein synthesis. However, an experiment in which cells were infected with equal particle numbers of the two viruses (1000 particles per cell) in the presence of actinomycin D showed that virion-associated shut off by HSV-140 was significantly reduced compared to that of wt virus (results not shown). This could be due to inefficient presentation by HSV-140 of the virion host shutoff function encoded by gene UL41 (K.-wong et al., 1988; McGeoch et al., 1988), or slight alterations in the composition or structure of HSV-140 virus particles might reduce their efficiency of cellular adsorption or penetration.

**Discussion**

The predicted amino acid sequence of VZV gene 62, which encodes VZV 140K, shows strong homology to Vmw175 of HSV-1 (McGeoch et al., 1986). Like Vmw175, VZV 140K is a potent trans-activator of gene expression in transfection assays (Everett & Dunlop, 1984; Everett, 1984; Inchauspe et al., 1989). Previous studies have shown that VZV 140K can complement the growth of HSV-1 viruses with ts and deletion mutations in Vmw175 (Felser et al., 1987, 1988). By construction of a virus in which the VZV 140K coding sequences replace those of Vmw175 we have confirmed and extended the previous observations on the relatedness of the two proteins. The results graphically illustrate the functional similarities of Vmw175 and VZV 140K since recombinant virus HSV-140 propagates in normal cell lines. Its failure to form clearly defined plaques may be a consequence of slight alterations in the balance of viral gene expression which would be difficult to define. Similarly, if one of the proteins involved in viral DNA replication were aberrantly expressed during HSV-140 infection it would not be easily detected by analysis of viral polypeptide expression, yet could account for the delayed and reduced levels of HSV-140 DNA replication. It is likely that the reduced expression of certain late proteins is a consequence of less efficient DNA replication and this in turn may explain the slower appearance of c.p.e. in HSV-140-infected cells. These results show that the properties of VZV 140K and Vmw175 are not indistinguishable but they are largely interchangeable.

The multiplicity dependence of HSV-140 gene expression is reminiscent of other HSV-1 variants with mutations in Vmw65 and Vmw110 (Stow & Stow, 1986; Sacks & Schaffer, 1987; Ace et al., 1989; Everett, 1989). Since one of the phenotypes of the Vmw65 virus mutant is synthesis of reduced amounts of Vmw110 and since the synergistic activation of gene expression by the combination of Vmw110 and Vmw175 observed in transfection assays (Everett, 1984; Quinlan & Knipe, 1985; Gelman & Silverstein, 1985) does not appear to occur with VZV 140K and Vmw110 (our unpublished data), it is intriguing to speculate that the multiplicity dependence of HSV-140 gene expression might be a reflection of this lack of synergy.

The regulation of VZV 140K expression during HSV-140 infection is also of interest since it accumulates to high levels as infection proceeds, which is in contrast to the relatively low levels of accumulation of Vmw175 during wt virus infection. Therefore, VZV 140K is apparently unable to effect the repression of IE3 gene expression which is normally a function of Vmw175. Repression of the IE3 promoter by Vmw175, which requires the presence of a specific Vmw175 DNA-binding sequence at the cap site of the IE3 promoter (Muller, 1987; Roberts et al., 1988), can be reproduced in transfection assays (OHare & Hayward, 1985, 1987). Mutants of the Vmw175 protein that fail to bind to an IE3 cap site probe are unable to repress the IE3 promoter (DeLuca & Schaffer, 1988; Paterson & Everett, 1988b). The failure of VZV 140K to repress the IE3 promoter during HSV-140 infection is consistent with data which show that VZV 140K does not repress the IE3 promoter in transfection assays (Disney et al., 1990) and does not bind to the IE3 cap site region (our unpublished data). We do not yet know whether the other IE genes are also overexpressed in HSV-140 infections, but if not it
suggests that the mechanism of their repression may differ from that of IE3. If this is the case, then HSV-140 may be a useful tool with which to study the mechanism of IE gene repression.

In conclusion, our results demonstrate that VZV 140K is able to complement, to a great extent, for loss of Vmw175 function in HSV-1. The high levels of expression of VZV 140K in HSV-140-infected cells should permit a more detailed analysis of the biochemical properties of the protein.

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