A prominent serine-rich region in Vmw175, the major transcriptional regulator protein of herpes simplex virus type 1, is not essential for virus growth in tissue culture

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Herpes simplex virus type 1 (HSV-1) encodes five immediate early (IE) genes of which at least three are involved in the transcriptional regulation of later classes of viral genes. Perhaps the most important of these regulatory proteins is Vmw175, a nuclear phosphoprotein of 1298 predicted amino acid residues. In the absence of functional Vmw175 the virus fails to activate early or late genes or to repress IE gene expression. All viruses of the sub-family alphaherpesvirinae encode polypeptides that are closely related to Vmw175. Mutational studies have shown that regions of homology within this family of gene regulators are generally of functional importance. One of the most striking conserved stretches of amino acid sequence is a run of serine residues followed by a highly acidic region in the amino-terminal fifth of the polypeptide. We have constructed an HSV-1 virus which lacks this serine-rich run within Vmw175. Surprisingly, the virus was viable in tissue culture cells and expressed apparently normal amounts of viral polypeptides. In plaque assays it was very slightly temperature-sensitive and, depending on the state of the host cells, could generate plaques with a syncytial morphology. The mutant protein was able to bind to DNA in a manner indistinguishable from that of the wild-type polypeptide. We conclude that despite its conservation in all of the alphaherpesvirinae so far sequenced, the serine-rich homology is not important for virus growth in tissue culture.

Introduction

Herpes simplex virus type 1 (HSV-1) has a linear double-stranded DNA genome of about 150 kb which encodes at least 70 distinct genes (McGeoch et al., 1988). These genes can be grouped into three broad classes depending on their temporal regulation during infection in tissue culture (for reviews see Wagner, 1985; Everett, 1987). The five immediate early (IE or α) genes can be transcribed in the absence of de novo viral protein synthesis by the host RNA polymerase II (Costanzo et al., 1977). Efficient use of the IE promoters requires recognition of an IE-specific regulatory sequence by a host transcription factor (probably NFIII or OTF-1) in association with a component of the virus particle, Vmw65 or α-TIF (Campbell et al., 1984; McKnight et al., 1987; Preston et al., 1988; O'Hare & Goding, 1988). Transcription from early promoters requires the expression of IE polypeptides while late gene transcription is most efficient only after the onset of viral DNA replication. Of the five IE gene products, that of IE-3 (Vmw175 or ICP4) is perhaps the most crucial for subsequent viral gene regulation as certain temperature-sensitive (ts) mutations which map in Vmw175 result in an absence of viral growth through failure to activate early and late promoters at the non-permissive temperature (Preston, 1979). In addition, lack of Vmw175 function results in loss of the autoregulation or repression of IE gene expression which occurs during normal infection. Thus Vmw175 is central to the regulation of all classes of viral genes and as such has been the focus for intensive study.

Vmw175 is a representative of a family of regulatory proteins that are conserved in the subfamily alphaherpesvirinae. It is a phosphorylated nuclear DNA-binding protein with an apparent Mr of 175K in SDS-polyacrylamide gels, and can be poly(ADP-ribosyl)ated in isolated nuclei (Powell & Purifoy, 1976; Pereira et al., 1977; Cabral et al., 1980; Hay & Hay, 1980; Preston & Notarianni, 1983). Phosphorylation of Vmw175 occurs at both serine and threonine residues (Faber & Wilcox, 1986a) and as pulse–chase experiments have indicated that phosphate groups can cycle on and off the polypeptide during infection (Wilcox et al., 1980) it is possible that phosphorylation plays some role in its regulatory activities. Vmw175 can be isolated in multimeric (principally dimeric) forms (Metzler & Wilcox, 1985) and has been purified almost to homogeneity (Kattar-Cooley & Wilcox, 1989).

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Comparison of the predicted amino acid sequence of Vmw175 with those of the related proteins expressed by varicella-zoster virus (VZV) (McGeoch et al., 1986), pseudorabies virus (PRV) (Vlcek et al., 1989) and equine herpesvirus (EHV) type 1 (Grundy et al., 1989) reveals two regions of high conservation (regions 2 and 4) interspersed with three other regions (1, 3 and 5) that have fewer similarities. Extensive mutagenic studies have revealed that region 2 contains sequences that are essential for Vmw175 functions in early gene activation and IE gene repression both during virus infection and in short term transfection assays using isolated genes and promoter targets (Preston, 1979; Davison et al., 1984; DeLuca & Schaffer, 1987, 1988; Paterson & Everett, 1988a). The structure of region 4 is also important for these functions (Paterson & Everett, 1988a; Paterson et al., 1990). Mutations in both regions 2 and 4 can also affect the ability of Vmw175 to bind in vitro to DNA fragments which include the consensus recognition sequence ATCGTC (Faber & Wilcox, 1986b; Müller, 1987; DeLuca & Schaffer, 1988; Paterson & Everett, 1988b; Kattar-Cooley & Wilcox, 1989; Shepard et al., 1989). Binding to such a consensus sequence at the cap site of the IE-3 promoter has been directly implicated in the ability of Vmw175 to repress IE transcription both in transfection assays and during virus infection (Roberts et al., 1988; DeLuca & Schaffer, 1988) and binding to a similar site upstream of the gD promoter contributes to promoter activation in vitro (Beard et al., 1986; Tedder et al., 1989).

Apart from the highly conserved regions 2 and 4, there are other short sections of the Vmw175 polypeptide that are conserved in the three related gene products so far sequenced. The most striking of these is a run of serine residues followed by a short highly acidic block in region 1. Conservation of this region in HSV, VZV and PRV, and the fact that a stretch of serine residues might be a major target for phosphorylation, suggests that it may be important for Vmw175 function. This paper assesses this question by the construction of a mutant virus which lacks the serine-rich region. Surprisingly, the virus was viable in normal tissue culture cells. The mutant Vmw175 polypeptide was capable of activating early and late gene expression (although rather more slowly than the wild-type virus) and it could also repress IE gene expression. The mutant polypeptide was able to bind to the consensus ATCGTC binding sequence at the IE-3 cap site. We conclude that the serine-rich tract is not essential for virus growth in tissue culture.

**Methods**

*Viruses and cells.* HSV-1 Glasgow strain 17 syn* was the wild-type virus used in this study. Both the wild-type and the mutant virus I15HBC were routinely propagated and titrated in BHK clone 13 cells which were grown in Glasgow-modified Eagle's medium (GMEM) supplemented with 10% newborn calf serum and 10% trypose phosphate broth. M64A cells, derived from BHK TK- cells by biochemical transformation to TK* by transfection of a plasmid containing the intact HSV-1 TK and IE-3 genes, were grown in GMEM supplemented with 10% foetal calf serum. M64A cells express Vmw175 from integrated IE-3 gene copies and are essentially identical to the M65 cells described by Davidson & Stow (1985). They were used for the propagation and titration of HSV-1 mutant in1411, which has translational termination signals inserted into the IE-3 gene near the S' end of the Vmw175 coding sequence (Russell et al., 1987), and the IE-3 deletion mutant D30EBA (see text). Human foetal lung (HFL) cells were obtained from Flow Laboratories and were grown in GMEM supplemented with 10% foetal calf serum. 2-BHK cells were derived from BHK cells by passage and maintenance in Dulbecco's modified Eagle's medium containing 2.5% foetal and 2.5% newborn calf sera.

*Bacteria and plasmids.* All plasmids were maintained in Escherichia coli strain HB101. Plasmid p175 contains the complete IE-3 coding region linked to the simian virus 40 promoter and enhancer (Perry et al., 1986). The deletion mutant plasmids pdelI11 and pD1 were derived from p175 as described in the text.

*Preparation and analysis of viral DNA.* Infectious viral DNA was prepared from cell-released viral stocks by the method of Wilkie (1973). Viral DNA for Southern blot analysis of the genotype was prepared as total cellular DNA from infected cell monolayers (Stow et al., 1983). Viral DNAs were analysed by restriction enzyme digestion, agarose gel electrophoresis and Southern blotting (Southern, 1975) using nick-translated probes (Rigby et al., 1977). Viral DNA was also labelled with [32P]orthophosphate during growth in tissue culture. The labelled DNA was isolated, cut with restriction enzymes, separated by agarose gel electrophoresis and detected by autoradiography as described by Lonsdale (1979).

*Transfection of tissue culture cells and isolation of recombinant viruses.* Infectious DNA from viruses in1411 or D30EBA was mixed with calf thymus carrier DNA and mutant plasmid DNA linearized at the PstI site and transfected into M64A cells by the method of Stow & Wilkie (1976). Recombinant viruses were isolated by titrating the progeny virus from the transfection on M64A cells under agar. Individual plaques were picked and the viruses grown up in single wells of M64A cells in 24-well Linbro blocks. When extensive c.p.e. had occurred, total cellular DNA was prepared from each well and analysed for the presence of the desired mutation by Southern blotting. Viruses which carried the required mutation in both copies of IE-3 were plaque-purified three more times on M64A cells. All viral preparations were analysed by Southern blotting to confirm the presence of the mutation. After the initial isolation in M64A cells, it was found that virus I15HBC could be propagated on BHK cells. Because the genotype of I15HBC was rather unstable in M64A cells (perhaps because of recombination with the IE-3 gene copies in the cellular genome) it was plaque-purified once more in BHK cells before large scale stocks were prepared.

*Labelling of viral polypeptides and their analysis by SDS-polyacrylamide gel electrophoresis.* BHK cells (4 × 10⁵ cells in Linbro wells) were infected with wild-type and mutant viruses at multiplicities of 5 p.f.u. per cell. At the times indicated in the text, the cells were washed with phosphate-buffered saline (PBS) and 10 μCi of [35S]methionine (Amersham, > 800 Ci/mmol) in PBS was added. After labelling for 2 h, the cells were harvested in sample buffer and analysed by SDS–polyacrylamide gel electrophoresis as described by Marsden et al. (1978).

*Injection of cells for the preparation of labelled IE protein extracts.* BHK cells (4 × 10⁵ cells in 24-well Linbro dishes) were preincubated for 30 min in the presence of cycloheximide (100 μg/ml) and then infected.
with the wild-type or mutant viruses at a multiplicity of 5 p.f.u. per cell. After a 1 h adsorption period, the cells were re-fed with growth medium containing cycloheximide and incubated at 37 °C for a further 4 h. The cells were then washed three times with PBS, then once with PBS containing 2.5 μg/ml actinomycin D, and then incubated with 10 μCi [35S]methionine (Amersham, > 800 Ci/mmol) in 0.2 ml PBS plus actinomycin D at 37 °C for 90 min. The cells were then washed with PBS and harvested directly into boiling mix (100 μl). This infection and labelling procedure maximizes the production of the IE proteins of HSV-1 (Preston et al., 1978).

Preparation of nuclear extracts from infected cells. Nuclear extracts of infected cells were prepared by a modification of the procedure of Dignam et al. (1983). HeLa cells were infected with 5 p.f.u. per cell of wild-type and mutant viruses at either permissive or non-permissive temperature. Five h after a 1 h adsorption period the cells were scraped into PBS, pelleted and resuspended in 2 volumes of buffer A (10 mM-HEPES pH 7.9, 1.5 mM-MgCl₂, 10 mM-KCl, 0.5 mM-EDTA) due to the occasional variation of the amounts of nuclear extract used does not affect the formation of the Vmw175-specific complex. Vmw175 is present in a buffer containing 10 mM-Tris-HCl pH 7.6, 1 mM-EDTA, 0.5 mM-PMSF, 0.5 mM-DTT) for 30 min with frequent mixing. The nuclear debris was pelleted by centrifugation in the Sorvall SS34 rotor at 2000 r.p.m. for 10 min, using 1-5 ml reaction vials set in rubber adaptors for this rotor. The supernatant was discarded and then the nuclei were further compacted by spinning at 12000 r.p.m. for 20 min in the same rotor. Proteins were eluted from the nuclei by incubation on ice in 2 volumes of buffer C (20 mM-HEPES pH 7.9, 25% glycerol, 0.42 mM- NaCl, 1.5 mM-MgCl₂, 0.2 mM-EDTA, 0.5 mM-PMSF, 0.5 mM-DTT) for 30 min with frequent mixing. The nuclear debris was pelleted by centrifugation in the Sorvall SS34 rotor at 15000 r.p.m. for 30 min. The supernatants containing the nuclear DNA-binding proteins were frozen in dry ice/ethanol and stored at −140 °C. The Vmw175 contained in such extracts retained the ability to bind to DNA, without any loss of activity, over several months and freezing and thawing cycles.

Analysis of the DNA-binding capability of Vmw175 by gel retardation. The ability of Vmw175 to bind to the IE-3 cap site region was assayed using the conditions and probe described by Müller (1987). The 45 bp BamHI-Aval fragment spanning the IE-3 cap site was end-labelled by filling in with T4 DNA polymerase, separated on an 8% polyacrylamide gel, eluted and purified through a Sephadex G-50 column. Binding reactions were carried out for 20 min at the temperatures indicated in the text in 20 μl volumes containing 1 μg poly(dI).

Results

Construction and isolation of an HSV-1 strain 17 mutant with a large deletion in IE-3

We have previously described the construction and characterization of a large number of insertion and deletion mutations in a plasmid-borne copy of IE-3, which encodes Vmw175. In order to assess the biological significance of the effects of these mutations, it is important to transfer selected mutations from the plasmid into the viral genome. As a first step in this procedure, it was desirable to construct a virus derived from strain 17 that lacks a large portion of the Vmw175 coding region to serve as the parent virus in the recombinant experiments. The deletion mutant would not outgrow any recombinant progeny and rescue of the deletion would ensure inheritance of the desired mutation if it lay in the deleted region. Although deletion mutants of IE-3 are available in strain KOS (DeLuca et al., 1985), they have not been described in strain 17. We decided to construct a large deletion mutant in the strain 17 IE-3 coding sequences using plasmid pdel11, which has lost sequences from codons 83 to 1236. To minimize the problem of the selective advantage of wild-type virus, we used infectious DNA from virus in1141 (Russell et al., 1987) as the parental virus DNA for the deletion mutagenesis. Virus in1141 has a translational termination signal at codon 83 of the Vmw175 coding sequence and does not express Vmw175. The position of IE-3 in the HSV-1 genome, and the locations of the mutations used in this study are shown in Fig. 1.

Viral in1141 DNA was transfected into M64A cells with linearized pdel11 plasmid DNA and progeny virus harvested 4 days later. The resulting virus stock was titrated on M64A cells under agar. A number of plaque isolates were picked and inoculated into Linbro wells of M64A cells and 4 days later the cell-released virus from each was harvested and total cellular DNA prepared from the cell monolayer. This DNA was digested with BamHI and subjected to agarose gel electrophoresis and Southern blotting. Using nick-translated plasmid p175 (Perry et al., 1986) as a probe, the isolates were screened for those having the required deletion in the joint fragment BamHI k. One isolate with a deleted BamHI k fragment was further plaque-purified three times (checking the restriction pattern on each occasion) and then a stock of the mutant virus D30EBA was prepared. The DNA of this virus was further analysed and compared to that of the parental virus in1141 by the technique of Lonsdale (1979). The results after digestion of the viral DNA with BamHI are shown in Fig. 2. The BamHI k joint fragment of in1141 has been lost from D30EBA (lane 1), as has the terminal fragment BamHI y (which comigrates with BamHI z). There is a faint novel BamHI band of the expected size (4·3 kb) between fragments n and o in D30EBA. It was difficult to detect all the predicted restriction fragments of D30EBA in this manner probably because of variations in the numbers of ‘a’ sequences and other insertions in the deleted short repeat terminal and joint regions, and also because the
method necessarily results in lower yields of the terminal fragments. However, further analysis of D30EBA by digestion with HindIII, EcoRI and KpnI gave results entirely consistent with the predicted genome structure (data not shown; Paterson, 1989).

Due to recombination between D30EBA and the IE-3 sequences integrated into the M64A cell line, stocks of D30EBA contained approximately 0-001% to 0-01% of apparently wild-type virus. However, the presence of such recombinant genomes was not a serious problem for the uses of D30EBA described in this paper. When D30EBA was used to infect BHK cells, the pattern of viral polypeptide synthesis was very similar to that observed with the parent in1411 and other mutants with large deletions in IE-3 (DeLuca et al., 1985). There was an accumulation of the IE polypeptides (with the exception of Vmw175), and also the large subunit of ribonucleotide reductase, but there was no evidence of synthesis of other early or late proteins (data not shown).

Construction of a virus lacking the serine-rich region of Vmw175

Plasmid pD1 encodes a deleted IE-3 gene lacking codons 162 to 229 and includes an EcoRI linker at the site of the deletion (Fig. 1; Paterson & Everett, 1988a). This deletion spans the serine-rich and acidic region from codons 176 to 206, which is conserved in the corresponding PRV, EHV and VZV polypeptides. In order to construct a virus with an IE-3 gene lacking this domain,
linearized plasmid pD1 DNA was cotransfected with D30EBA viral DNA into M64A cells. As described above for the construction of D30EBA, the progeny virus were plated onto M64A cells and single plaques were screened by Southern blotting for transfer of the plasmid pD1 deletion into the viral genome. A number of candidate isolates were picked for further purification. After three rounds of plaque purification (and a further purification on BHK cells; see below) stocks of isolate I15HBC, which exhibited the expected restriction profile on Southern blots, were prepared. The genomic DNA of I15HBC was further characterized by the technique of Lonsdale (1979) (Fig. 3). The results clearly show that I15HBC (lane 2), in comparison with strain 17 (lane 1) and rescued and parental viruses (lanes 3 to 6), has the expected EcoRI restriction profile. The terminal fragment EcoRI k has been lost and novel fragments, migrating above EcoRI m and below EcoRI o, result from the EcoRI linker which replaces the deleted sequences in I15HBC.

**Virus I15HBC grows on a non-complementing cell line**

When the growth characteristics of I15HBC were investigated, it was surprising to find that the virus formed plaques on BHK cells although the plaques were somewhat smaller than those of strain 17. The plaquing ability of I15HBC was slightly temperature-sensitive (a threefold to sevenfold reduction in titre at 38.5 °C compared to 31 °C) and at the higher temperature it had a tendency to produce syncytial plaques. This latter effect was dependent on the stock of BHK cells used for titration and was not always observed. To test whether the syncytial plaque phenotype of I15HBC was due to the deletion in Vmw175, or to some other adventitious change in the viral genome, the wild-type IE-3 gene was recombined into I15HBC by cotransfection of viral I15HBC DNA with either pGX58 (containing XhoI fragment c; Everett, 1984) or the EcoRI-SalI fragment spanning the region -129 to +2041 of the IE-3 transcription unit. Rescued viruses were picked on the basis of a non-syncytial plaque morphology and then tested for their genome structure. Rescued viruses (rl-10 and r2-1 respectively) from both experiments contained the wild-type IE-3 gene, thus strongly suggesting that the syncytial phenotype of I15HBC was due to the deletion in Vmw175 (Fig. 3).

After it became clear that I15HBC was able to plaque on BHK cells, the virus was routinely propagated on this cell type. Such stocks of the virus had particle to p.f.u. ratios in the range of 200:1 which is slightly higher than those observed with similar stocks of strain 17. The plating efficiency of I15HBC was similar on BHK, 2·5BHK and HFL cells, which illustrates that unlike viruses unable to express functional Vmw110 (Stow & Stow, 1985; Everett, 1989) there was no cell type-dependent effect. However, the syncytial plaque morphology of I15HBC was particularly marked in HFL cells.

The growth efficiency of I15HBC relative to strain 17 in BHK cells was tested in a single-step growth curve experiment. The results (Fig. 4) illustrate that the yield of I15HBC is about 10-fold less over the time course of this experiment. Therefore, although the serine-rich region of Vmw175 is not essential, it does contribute to fully efficient viral growth.

**Viral polypeptide expression of I15HBC**

Viral polypeptide expression induced by I15HBC was compared to that of the wild-type virus in BHK cells in a time course experiment. Cells were infected as described in Methods and subsequently labelled with [35S]methionine from 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10 and 14 to 16 h post-adsorption. The labelled proteins were analysed by SDS-gel electrophoresis (Fig. 5). Compared to the wild-type virus, viral polypeptide synthesis by I15HBC is slightly delayed (which is consistent with its smaller plaques and reduced yields) but apparently normal amounts of all of the obvious viral polypeptides are eventually produced. These results are consistent with previous transfection experiments which showed that Vmw175 expressed by plasmid pD1 was located in the nucleus and could trans-activate the gD promoter (although about three times less efficiently than the wild-
Fig. 5. Viral polypeptide synthesis in BHK cells infected with 17 + and 115HBC. BHK cells were infected with strain 17 (a) and 115HBC (b) at a multiplicity of 5 p.f.u. per cell and labelled with [35S]methionine at various times thereafter. Lanes 1 to 7, mock-infected cells, and cells labelled at 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10 and 14 to 16 h after adsorption, respectively. The four most prominent high Mᵋ viral polypeptides are the major capsid protein, the large subunit of ribonucleotide reductase (RR1), glycoprotein gB and the major DNA-binding protein. Further down the gel Vmw65 is seen accumulating in large amounts late in infection.

Fig. 6. Virus I15HBC produces a Vmw175 polypeptide of reduced Mᵋ.

BHK cells were infected with strain 17, mock-infected and infected with r2-1 and I15HBC (lanes 1 to 4 respectively) in the presence of cycloheximide. After reversal of the cycloheximide block viral IE proteins were labelled and separated by SDS-gel electrophoresis. The IE proteins are identified on the left and the smaller Vmw175 produced by I15HBC is indicated on the right.

type protein) and repress the IE-3 promoter (Paterson & Everett, 1988a). We conclude that, in the background of the virus, the serine-rich homology region is not required for the efficient function of Vmw175 in either early and late gene activation or the repression of IE gene expression in tissue culture.

Experiments using lower multiplicities of infection showed that I15HBC (again unlike viruses with defects in Vmw110; Everett, 1989) did not have a multiplicity-dependent defect in viral gene expression (data not shown). Labelling of the viral IE polypeptides after reversal of a cycloheximide block revealed that I15HBC expressed a Vmw175 polypeptide which was approximately the expected size, some 6K smaller than the wild-type protein (Fig. 6).

Polypeptide Vmw75 expressed by I15HBC binds to the IE-3 cap site recognition sequence in vitro

Vmw175 is a sequence-specific DNA-binding protein which recognizes a number of different sequences of which many contain the consensus ATCGTC (Faber & Wilcox, 1986b; Beard et al., 1986; Michael et al., 1988). The sequence at the cap site of the IE-3 promoter is strongly bound by Vmw175 (Müller, 1987). We have previously confirmed these results, finding that while extracts from mock-infected cells give two complexed bands in gel retardation experiments, infected cell extracts form a third band which can be further retarded by addition of the anti-Vmw175 monoclonal antibody 58S (Paterson & Everett, 1988b). This Vmw175-specific complex is not formed by extracts from cells infected with inl411 (Paterson & Everett, 1988b) or D30EBA (see below). We have investigated whether a similar Vmw175-specific complex can be formed with extracts from cells infected with I15HBC. The results (Fig. 7)
show that whereas extracts from D30EBA-infected cells gave only the two host bands B and C (lane 1), extracts from I15HBC-infected cells (lane 2) and rescued viruses r1-10 and r2-1 (lanes 3 and 4) gave the Vmw175-specific band A. The band A produced with I15HBC-infected extracts could be further retarded with monoclonal antibody 58S (data not shown). The amount of this band formed with I15HBC extracts was reproducibly less than that formed with either wild-type or rescued virus extracts (data not shown and Fig. 7) and this correlated with lower amounts of Vmw175 as detected by ELISA (data not shown). However, as extracts were routinely made 5 h post-infection with all viruses, the lower amounts of Vmw175 in I15HBC-infected extracts may simply represent the rather slower onset of infection with this virus (Fig. 3). Alternatively, the lack of the serine-rich homology region may reduce the stability of Vmw175. Despite the reduced amounts of the I15HBC complex it was as thermostable as the complexes formed with the rescued virus extracts (compare panels a and b, Fig. 7). These results using Vmw175 prepared from cells infected with I15HBC are consistent with the results using extracts from cells transfected with plasmid pD1 (Paterson & Everett, 1988b).

These results strongly suggest that the serine-rich homology region does not contribute to the DNA-binding domain of Vmw175. In further support of this hypothesis, we have shown that a small proteolytic subfragment of Vmw175 can form a complex with the IE-3 cap site probe and that the size of this complex is unaffected by the deletion of the serine-rich tract (unpublished results).

Discussion

This report clearly demonstrates that a prominent serine-rich tract and the surrounding sequences which include a highly acidic region (Fig. 8) are not required for the function of Vmw175 during HSV growth in tissue culture. This conserved region extends from codon 176 to codon 206, which is entirely within the deletion in I15HBC (codons 162 to 229). There is strong evidence that this region is a target for the normal phosphorylation of Vmw175. A mutant polypeptide including only the first 171 codons is not phosphorylated while another expressing the first 251 codons is (DeLuca & Schaffer, 1988). However, an in-frame deletion mutant from which residues 185 to 309 have been removed can be phosphorylated (DeLuca & Schaffer, 1988). This suggests that either the six remaining serine residues between 176 and 184 can be phosphorylated in the absence of the rest of the conserved region, or that other sites in the protein (including threonine residues; Faber & Wilcox, 1986a) are also targets for phosphorylation.

Whatever the role of this conserved sequence in the phosphorylation of Vmw175, it is very surprising that it is not essential for the function of the protein. The region is conserved in the corresponding genes of all the alphaherpesviruses so far sequenced and it might be expected that such a strongly conserved feature would have an important function. Indeed, other conserved segments of Vmw175 such as regions 2 and 4 have been shown to be functionally important. This is further underlined by the observation that the amino acid residues that have been mutated in temperature-sensitive alleles of Vmw175 are precisely conserved in the alphaherpesvirus family (Paterson et al., 1990). Therefore, although amino acid conservation within the coding regions of related proteins may be a good indicator of important or functional domains, virus
I15HBC illustrates that this may not always be the case (at least for virus growth in tissue culture).

Shepard et al. (1989) have also investigated the effect of mutations in region 1 on the function of Vmw175. Using a truncated Vmw175 protein including only the first 774 amino acid residues, they found that further deletion of residues 31 to 210, 31 to 274, and 143 to 210 (all of which remove the serine-rich region) did not affect autoregulation or DNA-binding to ATCGTC consensus sites. However, these deletions reduced transactivation of the TK promoter by the truncated polypeptide, and the largest deletion inhibited DNA-binding to the non-consensus sites in the TK promoter (Shepard et al., 1989). These deletions generally remove more coding sequence than the deletion in I15HBC and since they are in a molecule already substantially deleted, their phenotype may not reflect that pertaining to the complete protein. Indeed, these authors cite unpublished work entirely in agreement with the data presented here (Shepard et al., 1989).

The cell growth-dependent syncytial plaque phenotype of I15HBC is potentially interesting. Rescue of the deletion by transfection with a DNA fragment internal to IE-3 and screening for normal plaque morphology strongly indicates that the syncytial phenotype is due to the altered Vmw175 in I15HBC. This could result from slight alterations in the amount of expression of the viral envelope proteins, which implies that the extent of phosphorylation of Vmw175 could affect its ability to activate different viral genes. Interestingly, another viable virus with a small deletion in Vmw175 between codons 209 and 236 had a similar phenotype of small syncytial plaques (Schroder et al., 1985). However, in this case the phenotype could not be attributed to the deletion in Vmw175.

Despite the results presented in this paper, which illustrate that the serine-rich region is not essential for virus growth in tissue culture, it seems inconceivable that such a highly conserved region within the alphaherpesvirinae has no role in the virus life cycle. It remains to be seen whether the I15HBC mutation affects other properties of the virus such as latency or pathogenesis.

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