Mutational Analysis of the Herpes Simplex Virus Type 1 Trans-inducing Factor Vmw65

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SUMMARY

The herpes simplex virus type 1 (HSV-1) polypeptide Vmw65 is a structural component of the virus particle and is also responsible for trans-induction of immediate early (IE) transcription. Functional domains of this polypeptide were investigated by constructing a series of 10 plasmids each with a 12 bp insertion in the gene encoding Vmw65. Plasmids were analysed for their ability to stimulate IE transcription in short term transfection assays, and the altered Vmw65 polypeptides were assayed for the ability to form an IE-specific protein–DNA complex (IEC) in vitro. A direct correlation was observed between stimulation of transcription and formation of IEC, strongly suggesting that IEC is an important intermediate in transcription activation. Plasmids were also tested for their ability to rescue the temperature-sensitive mutation in the HSV-2 assembly mutant ts2203, since marker rescue analysis indicated that this mutation maps within the gene encoding Vmw65. Five plasmids failed to rescue ts2203, thereby defining regions of Vmw65 required for virus assembly. The results show that distinct domains exist in Vmw65 for activation of transcription and assembly of virus.

INTRODUCTION

Herpes simplex virus (HSV) gene expression can be classified into three phases, immediate early (IE), early and late. The IE genes are the first to be transcribed after infection and the gene products (IE polypeptides) are essential for early and late gene expression (Honess & Roizman, 1974; Clements et al., 1977). A distinctive feature of HSV gene activation is the stimulation of IE transcription by the late polypeptide Vmw65, the major tegument protein otherwise referred to as trans-inducing factor (TIF) (Post et al., 1981; Batterson & Roizman, 1983; Campbell et al., 1984; Pellett et al., 1985). The trans-induction of IE gene transcription by Vmw65 is dependent on the cis-acting regulatory element TAATGARAT (where R represents a purine residue) which is located upstream of the mRNA cap sites of all IE genes (Mackem & Roizman, 1982a, b; Kristie & Roizman, 1984; Preston et al., 1984; Gaffney et al., 1985; Bzik & Preston, 1986; O'Hare & Hayward, 1987).

Polypeptide Vmw65 is the only viral protein required for recognition of TAATGARAT, since trans-induction can be achieved by transfection of plasmids which encode Vmw65 alone (Campbell et al., 1984; Pellett et al., 1985). It has recently been shown that Vmw65 interacts with cell factors, probably including nuclear factor III, to form a complex (IEC) which binds specifically to DNA sequences containing TAATGARAT (McKnight et al., 1987; O'Hare & Goding, 1988; Preston et al., 1988), and it is thought that IEC is an important intermediate in the induction of IE gene transcription. Thus, Vmw65 is involved in initiation of viral gene expression at a very early stage of infection.

Apart from its role in stimulating IE transcription, Vmw65 is an abundant viral structural protein, estimated to be present at approx. 1000 molecules per virion (Heine et al., 1974;
Roizman & Furlong, 1974). It is the major polypeptide of the tegument, and therefore would be expected to be important for the maintenance of virus structure.

To define functional domains of Vmw65 and hence obtain further information on its mode of action both as a transcription activator and as a structural protein, we have constructed a series of in-frame oligonucleotide linker insertion mutants in the gene that encodes the polypeptide. This approach was possible because the gene has been identified, cloned and its nucleotide sequence determined (Campbell et al., 1984; Dalrymple et al., 1985; Pellett et al., 1985). The results from an analysis of 10 mutants indicate that the ability of Vmw65 to form IEC correlates with its \textit{trans}-inducing activity, and that specific domains required for \textit{trans}-induction and correct virion assembly can be distinguished within the polypeptide.

\section*{Methods}

\textbf{Cells and viruses.} BHK-21 (C13) cells (Macpherson & Stoker, 1962) were used throughout. The HSV type 2 (HSV-2) temperature-sensitive (ts) mutant ts2203 was derived from strain HG52.

\textbf{Plasmids.} Plasmid pIE4/5CAT was constructed by replacing the HSV-2 promoter in pLW2 (Gaffney et al., 1985) with the promoter and upstream regulatory fragments of the HSV-1 IE gene 4/5. The IE4/5 upstream fragment used was defined by \textit{EcoRI} and \textit{SalI} sites in plasmid pTKN7 (Preston et al., 1984) which represent nucleotides -402 and -240 respectively. The \textit{EcoRI} site was converted to a \textit{ClaI} site by insertion of an oligonucleotide linker (CATCGATG). The IE4/5 promoter was contained in a \textit{SalI} (converted from \textit{SmaI}/\textit{NruI}) fragment spanning nucleotides -69 to +99 in plasmid pS20TK (Murchie & McGeoch, 1982; Preston et al., 1984). The upstream and promoter fragments were ligated together at the \textit{SalI} site, and the resultant 330 bp \textit{ClaI}/\textit{NruI} product was inserted into pLW2 between the \textit{AccI} site and a filled-in \textit{BamHI} site. Thus the HSV-2 IE4/5 promoter in pLW2 was replaced by the upstream and promoter sequences of the HSV-1 IE4/5 gene, without the HSV-1 ori\textsubscript{s} sequences (Stow & McMonagle, 1983; Preston et al., 1984).

Plasmid pMC17 was constructed from pMC1, a plasmid that consists of a 2609 bp fragment defined by map coordinates 0-669 to 0-685 on the prototype HSV-1 genome (Campbell et al., 1984) cloned into pUC9 (Fig. 1). A 1521 bp \textit{EcoRV}/\textit{FokI} fragment of pMC1, containing the entire coding sequence for Vmw65, was cloned into the \textit{HincII} site of pUC9 to give pMC17 (Fig. 2). The \textit{HindIII}/\textit{EcoRI} fragment from pMC17 was inserted into the corresponding sites of the multilinker in pGEM2 (Promega Biotech, Madison, Wis., U.S.A.) to give pGEMTIF (Fig. 2). Plasmid pGEMTIF therefore contained the entire coding sequence of Vmw65 under the control of the \textit{BamHI} promoter. Insertion mutations of Vmw65 were introduced into pGEMTIF by exchanging DNA fragments of pGEMTIF containing coding sequence with corresponding fragments of the pMC1, in series of plasmids that contained the insertion (Fig. 2). Only those mutations between the unique \textit{ApaI} and \textit{SalI} restriction sites were introduced into pGEMTIF, and the resulting constructions were designated pGEMTIF.in(X).

Construction of insertion mutants. Plasmid pMC1 was cleaved with \textit{HaeIII} in the presence of ethidium bromide using conditions found empirically to produce a maximum of singly cut linear molecules (Fig. 1). A 0-22 kb \textit{HaeIII} fragment was ligated with a 50-fold molar excess of phosphorylated 12 bp \textit{BamHI} linker (CGCCGAATTCGCG). DNA ligase was inactivated by heating for 5 min at 60 °C and the DNA was digested with \textit{BamHI}. Linear molecules were resolved by electrophoresis on a 1\% agarose gel, eluted from the gel and religated. Plasmids containing linker insertions were identified by analysis of small scale plasmid preparations. The locations of linker insertion sites were determined by restriction endonuclease mapping.

Transfections of BHK cells and chloramphenicol acetyltransferase (CAT) assay analysis. Monolayers of 10\textsuperscript{6} BHK cells were co-transfected at 37 °C with 1 \textmu g of insertion mutant plasmid and 1 \textmu g of pIE4/5CAT by the calcium phosphate precipitation method (Campbell et al., 1984). The total amount of DNA present was adjusted to 3-0 \textmu g by the addition of pUC9.

CAT assays were carried out as described by Gorman et al. (1982). The radioactivity in the substrate and the acetylated product spots was determined by scintillation counting to calculate the percentage conversion of substrate to product. The protein concentration of each extract was determined (Bradford, 1976) and the percentage conversion per mg of protein calculated. The amount of extract tested was varied to ensure that the linear response range of the assay was used.

In \textit{vitro} transcription of pGEMTIF.in plasmids and translation of RNA templates. The pGEMTIF.in plasmids were cleaved with \textit{EcoRI} before the transcription reaction. In \textit{vitro} transcription was performed using the Riboprobe system (Promega Biotech) following the manufacturer's protocol and incubating 1 \textmu g of plasmid DNA, 0-5 mM-G(5')ppp(5')G (Pharmacia) and 0-4 mM of each nucleoside triphosphate in a total volume of 25 \textmu l at 37 °C for 1 h.

In \textit{vitro} translation was carried out by addition of 2-5 \textmu l of transcription reaction mixture to 20 \textmu l of rabbit
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Fig. 1. A map of the HSV-1 genome in the prototype orientation, and position of Vmw65 (upper section). UL and US are the long and short unique regions bounded by repeated sequences represented by open boxes. pMC1 contains the HSV-1 sequence between SalI and XhoI sites cloned into pUC9. The lower section shows the construction of insertion mutations within the gene encoding Vmw65, as described in Methods.

Fig. 2. Construction of templates for in vitro transcription. (i) pMC17 was constructed by insertion of a 1521 bp EcoRV/FokI fragment from pMC1 into the HincII site of pUC9. (ii) The 1521 bp fragment was excised from pMC17 with HindIII and EcoRII and cloned between the HindIII and EcoRII sites of the pGEM2 transcription vector, to give pGEMTIF. (iii) Construction of insertion mutants. A 1048 bp ApaI/SalI fragment from the pMC1.in plasmids was excised and exchanged with the corresponding fragment in pGEMTIF to give the pGEMTIF.in series.
reticulocyte lysate (Amersham). Duplicate samples were incubated for 90 min at 30 °C either in the presence or in the absence of 25 to 50 μCi [35S]methionine (sp. act. > 800 Ci/mmol) in a 25 μl reaction mixture. Non-radioactive samples were stored at −70 °C for use in gel retardation assays. The radiolabelled translation mixture was processed for SDS-PAGE as described by Preston (1979).

**Gel retardation analysis of mutant polypeptides.** Formation and analysis of protein–DNA complexes was performed as described by Preston et al. (1988). A 77 bp DNA fragment, containing IE4/5-specific sequences located between SmaI sites at −362 and −297 relative to the transcription start site plus extra nucleotides due to the presence of BamHI and BglII linkers, was end-labelled with 32P and purified from a polyacrylamide gel. Reaction mixtures contained the following components: 10 mM-HEPES pH 7.9, 0.6 mM-dithiothreitol, 2.3 mM-MgCl2, 85 mM-NaCl, 0.1 mg/ml bovine serum albumin, 4 μg poly(dI)-poly(dC), 0.2 ng 32P-3' end-labelled fragment, 5.0 μg mock-infected HeLa cell nuclear extract and 5 μl reticulocyte lysate which had been incubated with Vmw65 mRNA synthesized in vitro. Incubation was at 25 °C for 30 min, and reaction mixtures were loaded directly onto a 3.5% polyacrylamide gel. After electrophoresis for 3.5 h at 160 V, the gel was dried and exposed for autoradiography.

**Marker rescue analysis.** Marker rescue was carried out as described previously (Stow et al., 1978). DNA (0.2 μg) of the HSV-2 ts mutant ts2203 and linearized plasmid DNA (0.5 μg) was co-transfected onto monolayers of 106 BHK cells by the calcium phosphate method (Campbell et al., 1984). The total amount of DNA added was adjusted to 3.0 μg using calf thymus DNA. After 3 to 4 h the monolayers were treated with 25% dimethyl sulphoxide and incubated at 31 °C for 3 days. Virus was harvested and titrated at the permissive temperature (PT) of 31 °C and the non-permissive temperature (NPT) of 38.5 °C. The efficiency of marker rescue was determined from the formula (titre at NPT/titre at PT) × 100.

### RESULTS

**Isolation of insertion mutants within the Vmw65-coding region**

Plasmid pMC1 contains the gene encoding Vmw65 and no other complete reading frames from HSV-1 of acceptable codon usage (Dalrymple et al., 1985; Pellelt et al., 1985). The approach used to mutagenize Vmw65 was to construct a series of mutants each with a 12 bp BamHI oligonucleotide inserted into pMC1 at a different location within the Vmw65-coding region, as described in Methods (Fig. 1).

Plasmids with insertions at 10 of the 17 HaeIII sites within the gene were isolated. A summary of the mutants obtained, together with their positions and the resultant amino acid changes, is shown in Fig. 6.

**Trans-inducing activity of insertion mutants**

Previous studies have shown that cloned HSV DNA fragments which encode Vmw65 can stimulate transcription from IE promoters in short term co-transfection assays (Campbell et al., 1984). This approach was used to analyse the trans-inducing phenotype of insertion mutants, and the results of a representative experiment are shown in Fig. 3. When a plasmid containing the HSV-1 IE4/5 promoter and upstream regulatory sequences linked to the CAT gene (pIE4/5CAT) was transfected alone into BHK cells a basal level of CAT expression from this promoter was detected (Fig. 3, lane 5). When pMC1 was included in the transfection, however, CAT activity increased by six- to eightfold (Fig. 3, lane 1). The insertion in HaeIII site 4 (in4) did not affect trans-induction (Fig. 3, lane 2), whereas the insertion in HaeIII sites 8 (in8) and 14 (in14) (Fig. 3, lanes 3 and 4 respectively) essentially abolished the effect. A summary of the results from three to six experiments using the complete series of plasmids is shown in Table 1.

Six of the mutant plasmids (in2, in4, in7, in11, in15 and in17) were indistinguishable from pMC1 in their ability to stimulate IE transcription, showing that insertions of four amino acids can be tolerated in certain regions of Vmw65. Three mutants, in8, in9 and in14, were reduced by 90% or more in their trans-inducing ability and thus represent plasmids in which the insertion caused a strong impairment of activity, within the limits of sensitivity of the assay. Any residual activity in in8 and in14 amounted to no more than 5% of the pMC1 level. One plasmid, in12, gave an activity which was reduced by about 50%.

In an additional co-transfection experiment plasmid pIE2TK, which contains the HSV-1 IE gene 2 promoter and upstream regulatory region linked to the HSV-1 thymidine kinase (TK)-coding sequences, was substituted for pIE4/5CAT and transfections were carried out at both 31
Fig. 3. Trans-induction of IE transcription by mutant plasmids. CAT assays were carried out on extracts of BHK cells co-transfected with pIE4/5CAT plus wt plasmid (lane 1), mutant plasmids in4 (lane 2), in8 (lane 3) or in14 (lane 4), or pUC9 (lane 5). The positions of 3-acetyl chloramphenicol (3-AC.CAM) and chloramphenicol (CAM) are indicated.

Table 1. Trans-inducing activity of Vmw65 mutants

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Trans-inducing activity (%)*</th>
</tr>
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<tbody>
<tr>
<td>pMC1</td>
<td>100</td>
</tr>
<tr>
<td>in2</td>
<td>88 (13)</td>
</tr>
<tr>
<td>in4</td>
<td>104 (34)</td>
</tr>
<tr>
<td>in7</td>
<td>78 (19)</td>
</tr>
<tr>
<td>in8</td>
<td>4 (1)</td>
</tr>
<tr>
<td>in9</td>
<td>10 (5)</td>
</tr>
<tr>
<td>in11</td>
<td>112 (13)</td>
</tr>
<tr>
<td>in12</td>
<td>46 (13)</td>
</tr>
<tr>
<td>in14</td>
<td>3 (4)</td>
</tr>
<tr>
<td>in15</td>
<td>111 (13)</td>
</tr>
<tr>
<td>in17</td>
<td>105 (30)</td>
</tr>
<tr>
<td>No pMC1</td>
<td>0</td>
</tr>
</tbody>
</table>

* The stimulation of expression from the IE4/5 promoter is given as a percentage of that obtained in parallel experiments with pMC1. The mean of at least three independent determinations was calculated and the standard error is presented in parentheses.

and 38.5°C. The effect of the insertion mutant plasmids on TK expression from pIE2TK (results not shown) was analogous to the effect on CAT expression from pIE4/5CAT at either temperature, showing that the nature of the ‘reporter’ gene or IE promoter did not affect the conclusions and that none of the insertions resulted in a protein ts for trans-activation.

Thus the 10 insertion mutants exhibited trans-inducing phenotypes ranging from no alteration to abolition of activity.

Formation of a protein–DNA complex directed by Vmw65 synthesized in vitro

Vmw65 associates with cellular factors to form a complex, IEC, which binds specifically to TAATGARAT elements (McKnight et al., 1987; Preston et al., 1988). The supposition inherent in these findings, that IEC is important for IE gene induction, was investigated by correlating the ability of mutated forms of Vmw65 to produce the complex with their trans-inducing phenotypes. Wild-type (wt) and mutant Vmw65 were synthesized by coupled in vitro transcription and translation, using the pGEM system. The products were incubated with HeLa
cell nuclear extracts and IEC was detected by analysis of retardation of the electrophoretic mobility of a 77 bp DNA fragment containing the regulatory region, including TAATGARAT, of IE gene 4/5. Fig. 4 shows that Vmw65 is the mRNA-dependent protein synthesized in vitro using the wt and mutant plasmid templates. This result rules out the possibility that frameshift mutations, resulting from deletion of a terminal nucleotide during cloning, occurred during construction of the insertion mutants.

The formation of IEC by Vmw65 synthesized in vitro was analysed by gel retardation assays (Fig. 5). When Vmw65 extracted from virus particles was added to reaction mixtures containing HeLa cell nuclear extract and the $^{32}$P-labelled 77 bp DNA fragment, the slowly migrating complex IEC was formed (lane 1). Addition of reticulocyte lysate containing Vmw65 synthesized in vitro, in place of HSV virion extract, also resulted in the production of IEC (lane 2), whereas this complex was not formed when reticulocyte lysate incubated without mRNA was added (lane 3). The complex labelled HC3, present in all lanes, is the result of HeLa cell nuclear proteins binding to TAATGARAT (Kristie & Roizman, 1987; O'Hare & Goding, 1988; Preston et al., 1988), and the band in lane 3 which migrated more rapidly than IEC probably represents the previously described complex HC4, which results from non-specific binding of HeLa cell proteins to DNA fragments (Preston et al., 1988). HC4 was observed only when reticulocyte lysate without transcription mixture was used in the gel retardation assay, presumably because pGEM DNA from the transcription mix acts as an additional non-specific
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Fig. 5. Gel retardation assay using in vitro synthesized Vmw65 products. A 77 bp DNA fragment containing the IE4/5 regulatory sequence was incubated with HeLa cell nuclear extract and virion extract (lane 1), rabbit reticulocyte lysate (lane 3), lysate containing Vmw65 synthesized in vitro (lane 2), or lysate containing mutant proteins synthesized in vitro from pGEMTIF: in7, -8, -9, -11, -12, -14 and -15 (lanes 4 to 10 respectively). The positions of the complexes IEC, HC3 and HC4 are indicated.

competitor. In control experiments it was found that HeLa cell nuclear extract was necessary for the formation of IEC; thus the reticulocyte lysate did not contain significant amounts of the cell polypeptide that interacts with Vmw65 (results not shown).

When mutant Vmw65 synthesized in vitro was used in the gel retardation assay, a strong correlation between complex formation and trans-inducing activity was observed. Mutants in7, in11 and in15 all formed IEC (lanes 4, 7 and 10 respectively) and were also capable of stimulating transcription from IE promoters. Similarly mutants in8, in9 and in14 did not form IEC (lanes 5, 6 and 9 respectively) and did not trans-induce IE transcription. The correlation is highlighted by in12 which had intermediate trans-inducing activity and formed the IEC but in lesser amounts (lane 8). These results strongly suggest that the production of IEC is an important stage in the stimulation of transcription from IE promoters.

Marker rescue of ts2203 by insertion mutants

To investigate domains of Vmw65 important for the correct assembly of virions, mutant plasmids were analysed for the ability to rescue the ts mutation in the HSV-2 mutant ts2203. This
Table 2. Marker rescue of ts2203 by mutant plasmids

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Marker rescue (NPT/PT \times 100)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BglII i</td>
<td>22.0</td>
</tr>
<tr>
<td>BglII i (ts13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pMC1</td>
<td>2.6</td>
</tr>
<tr>
<td>in2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in7</td>
<td>0.2</td>
</tr>
<tr>
<td>in8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>in12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in14</td>
<td>0.77</td>
</tr>
<tr>
<td>in15</td>
<td>1.2</td>
</tr>
<tr>
<td>in17</td>
<td>1.8</td>
</tr>
<tr>
<td>No pMC1</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

* The ability of mutant plasmids to rescue the ts lesion in HSV-2 ts2203 is expressed as the percentage of resulting non-ts recombinants in the total virus progeny. The results are a mean of two independent determinations.

The mutation in ts2203 did not affect the production of IE polypeptides at 38.5 °C over a range of input m.o.i. (results not shown), and cloned BglII i from ts13 was as effective as BglII i from wt HSV-2 in trans-inducing IE promoters at 31 or 38.5 °C in transient expression assays (results not shown). The mutation in ts2203 therefore appears to have no effect on IE transcription, and further studies have revealed a block in virus assembly at 38.5 °C (F. H. Ramsay & V. G. Preston, unpublished data).

Intertypic marker rescue was carried out using pMC1 and the insertion mutants, on the assumption that plasmids which failed to rescue ts2203 had defects incompatible with the proper assembly of virions, provided the ts2203 mutation is in the HSV-2 counterpart of Vmw65. It was found that pMC1 rescued ts2203 although, as expected, the efficiency was lower than with HSV-2 fragments (Table 2). Five mutants (in2, in7, in14, in15 and in17) rescued ts2203, whereas five (in4, in8, in9, in11 and in12) did not. One conclusion from this experiment is that the ts2203 mutation must lie within the coding sequences of the HSV-2 homologue of Vmw65, since this is the only gene affected by the oligonucleotide insertions in4, in8, in9, in11 and in12. Furthermore, the failure of these plasmids to rescue ts2203 indicates that the insertions affect regions essential for virus assembly. The interpretation of positive rescue is less clear, since two mechanisms might operate. Such a result may have been obtained because the insertion does not affect virion assembly, but it is also possible that the insertion lies sufficiently far from the ts2203 mutation that intragenic recombination, without incorporation of the mutation into viral DNA, could account for the observations. The latter argument does not apply to in7, since both in4 and in8 were unable to rescue ts2203, nor to in2 if the ts2203 mutation is truly at the 5’ end of the coding sequences.

Thus, of the five sites at which insertions prevent rescue only two (in8 and in9) are loci of major importance for both virus assembly and trans-induction of IE transcription. The features of Vmw65 required for virion assembly are not invariably the same as those involved in transcription activation.
Fig. 6. A summary of the phenotypes of mutated Vmw65 polypeptides. A + sign denotes that the polypeptide has wt phenotype, − indicates that the function has been abolished, ± represents intermediate activity, and ND means that activity was not determined. The inserted amino acids, determined by the reading frame of the insertion site, are (a) ARIR, (b) ADPR or (c) RGSA. The scale bar represents a length of 50 amino acids.

DISCUSSION

Insertion of a 12 bp oligonucleotide into coding sequences is a convenient method for making relatively small changes in the amino acid sequence of a protein. Three different insertions were possible in the experiments described here, depending upon the reading frame at the site of insertion, as shown in Fig. 6. Alteration in the properties of a protein could result from local or overall physical distortion or charge imbalance, but in a study of the HSV-1 IE polypeptide Vmw110 it was concluded that the properties of the mutated protein correlated more strongly with the position than with the nature of small insertions (Everett, 1987). A summary of the phenotypes of mutant plasmids is presented in Fig. 6.

Three mutants, in8, in9 and in14, were strongly reduced in their ability to trans-induce IE transcription, and since HaeIII sites 8 and 9 are only 15 bp apart it is probable that these three mutants define only two important regions. If the mutations exert their effects only at the site of insertion rather than by disruption of gross secondary or tertiary structure, it is possible that the regions defined by in8/9 and in14 are in close proximity and form a single domain in the properly folded protein. No striking features of the amino acid sequence at the sites of these insertions is obvious at present, and a computer-assisted search of the NBRF database did not reveal any proteins with clearly meaningful homologies to these regions. There is, however, a region of nine amino acids (ELRAREESY) only three amino acids upstream from the in8 site which is strongly conserved between Vmw65 and its varicella-zoster virus homologue (Dalrymple et al., 1985). In addition to the in8/9 (amino acids 172 and 177) and in14 (amino acid 379) regions, the region between amino acids 411 and 453 is also known to be important for trans-induction, since deletion back to a SmaI site (i.e. amino acid 453) has no detectable effect, whereas a frameshift insertion at a SalI site (amino acid 411) abolishes trans-induction (C. M. Preston & M. E. M. Campbell, unpublished observations; Triezenberg et al., 1988).

Proteins containing insertions at sites 8, 9 and 14 all failed to form IEC, suggesting that these mutations prevented Vmw65 from interacting with the cellular polypeptide(s) contained in IEC. It may be, therefore, that one or more of these sites defines amino acids involved in the protein–protein interactions that are essential for the production of IEC (Preston et al., 1988). The observation that the ability to trans-induce IE transcription correlates well with the formation of IEC provides evidence that the complex is an essential intermediate in IE gene activation. Other phenotypes of Vmw65 mutants can be envisaged; for example, a protein unable to mediate trans-induction might form an aberrant IEC that could not interact correctly with additional transcription factors.

The experiments described here show that the structural requirements of Vmw65 for transcription activation and virion assembly differ. The mutants in4 and in11 define sites which are important for virion structure but not trans-induction, and the mutation of ts2203 also falls into this category. Presumably these observations reflect the fact that Vmw65 is a multifunctional protein which interacts with virus structural proteins during virion assembly,
whereas it is complexed with one or more host cell proteins when acting as a trans-inducing factor. Insertions that affect both functions may disrupt a domain important for both properties, or they may change severely the secondary and tertiary structure, resulting in a grossly altered protein. Mutant in14 is unable to trans-induce but can rescue ts2203, suggesting that it is defective only for trans-induction. In turn, this interpretation implies that viable viruses containing the in14 mutation could be constructed, and thus that trans-induction is not essential for virus growth. It should be noted, however, that site 14 is distal to the proposed location of the ts2203 mutation, and therefore that recombination between the two mutations could occur. This interpretation cannot be ruled out, as it can in the case of in2 and in7, since both downstream insertions (in15 and in17) rescue successfully. Attempts to isolate virus mutants containing the in2, in7, in14 and in15 insertions are currently in progress.

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