The herpes simplex virus protein Vmw65 can *trans*-activate both viral and cellular promoters in neuronal cells

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Transcription of the herpes simplex virus (HSV) immediate-early (IE) genes in lytic infection is dependent upon the formation of a complex between the cellular transcription factor Oct-1 and the HSV virion protein Vmw65. This complex then binds to the TAATGARAT sequence in the IE promoters and *trans*-activates the IE genes. Following infection of neuronal cells such as the C1300 neuroblastoma cell line, however, the viral (IE) genes are not transcribed and the lytic cycle is aborted at an early stage. We show here that the cellular factors necessary to form a *trans*-activating complex with Vmw65 are present in C1300 cells and that *trans*-activation of both viral and cellular promoters by Vmw65 can be observed in these cells. In contrast with permissive cells, however, *trans*-activation is only observed in C1300 cells at a high concentration of the target viral promoter and not at a low concentration of the target promoter, regardless of the amount of Vmw65 transfected. The significance of these effects for the regulation of latent infection and cellular gene expression in neuronal cells is discussed.

INTRODUCTION

Herpes simplex virus (HSV) establishes long-lived infections in ganglionic sensory neurons (for reviews, see Roizman & Sears 1987; Latchman, 1990). Although such infections are asymptomatic, they provide a reservoir of virus which can repeatedly emerge and carry out a full lytic cycle in susceptible epithelial cells. It is now well established that the viral lytic cycle is aborted in neuronal cells due to a failure to express the viral immediate-early (IE) genes, their corresponding mRNAs and proteins being undetectable in latently infected ganglia of mice (Stevens *et al.*, 1987) and humans (Croen *et al.*, 1987). Since these proteins are crucial for the induction of the viral early and late genes (Watson & Clements, 1980) their absence ensures that the lytic cycle will not proceed.

Interestingly, IE gene expression in the lytic cycle is dependent upon the interaction of cellular transcription factors, such as Sp1 and the octamer binding protein Oct-1, with binding sites in the IE promoters (Jones & Tjian, 1985; O'Hare & Goding, 1988). The interaction of these factors with their corresponding binding sites produces a moderate level of IE promoter activity. Maximal activity of IE promoters is dependent, however, on a viral protein, Vmw65, which is carried in the incoming virion and *trans*-activates the IE promoters. This *trans*-activation is also achieved, however, by interaction with cellular factors. Thus Vmw65 forms a complex with Oct-1 and other cellular factors (Gerster & Roeder, 1988; O'Hare & Goding, 1988; Preston *et al.*, 1988). This complex then binds to the TAATGARAT sequences in the IE promoters with much higher affinity than does Oct-1 alone, resulting in *trans*-activation of the promoter (Campbell *et al.*, 1984; O'Hare & Goding, 1988; Preston *et al.*, 1988).

The strong dependence of both basal IE promoter activity and its *trans*-activation on cellular factors suggests that the failure of IE gene expression in neuronal cells may reflect differences in these factors in such cells. Since such differences are likely to reflect corresponding differences in cellular gene regulation in

neuronal cells, a study of the regulation of HSV IE promoters in neuronal cells may provide information both on the regulation of latent infection and on the processes regulating cellular gene transcription in these cells. Unfortunately, the amounts of material available from neuronal cells *in vivo* are insufficient for such a study.

To overcome this problem, we have investigated the regulation of IE gene expression in C1300 cells, an immortal mouse cell line originally isolated from a neuroblastoma (Augusti-Tocco & Sato, 1969). These cells are non-permissive for HSV (Vahlne & Lycke, 1977, 1978) and fail to synthesize the viral IE proteins following infection (Ash, 1986). We have recently shown that this is due to a failure of viral IE gene transcription after infection (Kemp & Latchman, 1989). This lack of IE gene transcription is caused by a cellular transcription factor, specific to neuronal cells, which binds to the TAATGARAT motif in the IE promoters; removal of this factor by addition of excess TAATGARAT elements resulted in an increase in IE promoter activity in C1300 cells (Kemp *et al.*, 1990). Binding of this factor to the TAATGARAT sequence is therefore responsible for the very low basal activity of IE promoters in C1300 cells. In view of the critical role of the TAATGARAT sequence in *trans*-activation by Vmw65, we have therefore investigated whether the presence of this neuronal protein prevents *trans*-activation from occurring in C1300 cells.

MATERIALS AND METHODS

Transfection

Transfections of plasmid DNA into either C1300 cells (Augusti-Tocco & Sato, 1969) or BHK-21 cells (clone 13; Macpherson & Stoker, 1962) were carried out by the calcium phosphate procedure, as described by Gorman (1985). Transfections employed either 1 µg or 5 µg of test plasmid DNA (as indicated in the Figure legends) per 2 × 10⁶ cells on a 90 mm plate. The total amount of DNA added to each plate was

Abbreviations used: HSV, herpes simplex virus; IE genes, immediate-early genes; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus.

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equalized with carrier DNA (total salmon sperm or pAT 153 plasmid). Following transfections, cells were harvested and the protein content was determined by the method of Bradford (1976). Samples equalized for protein content were assayed for chloramphenicol acetyltransferase (CAT) activity by the method of Gorman (1985).

Nuclear run-on assays

Nuclear run-on assays to measure transcription were carried out as previously described (Patel *et al.*, 1986) using nuclei prepared from transfected cells. The labelled products of the nuclear run-on assay were used to probe replicate dot-blots on to which 5 µg of the appropriate plasmid DNAs had been spotted.

Plasmid DNAs

The IE-CAT construct contains the sequences from -330 to +33 of the HSV-1 IE three-gene promoter driving expression of the CAT gene (Stow *et al.*, 1986). The RSV-CAT construct contains the long terminal repeat promoter of Rous sarcoma virus (RSV) driving expression of the CAT gene (Gorman *et al.*, 1982). The pMCI plasmid contains the full coding sequence for the Vmw65 protein driven by its own promoter (Campbell *et al.*, 1984). The pF construct contains a 63 bp fragment from the IE4/5 promoter containing a single TAATGARAT element and no other DNA-binding motifs. The U1 (Lund & Dahlberg, 1984) and U3 (Suh *et al.*, 1986) clones contain the full coding sequence of the respective human genes.

RESULTS

To study the effect of Vmw65 on IE gene expression in C1300 cells, we transfected these cells and HSV-permissive BHK cells with a construct (IE-CAT) in which the HSV IE three-gene promoter drives expression of the CAT gene (Stow *et al.*, 1986). Transfections were carried out using either 1 or 5 µg of IE-CAT DNA with or without 5 µg of DNA from plasmid pMCI, which encodes a functional Vmw65 protein.

In these experiments (Fig. 1), *trans*-activation of the IE promoter was observed, as expected, in BHK cells at both low and high concentrations of IE-CAT. In C1300 cells, the basal level of IE-CAT activity was much lower, as we have previously observed (Kemp *et al.*, 1990). However, *trans*-activation by Vmw65 was clearly observed in these cells at the higher concentration of IE-CAT. This indicates, therefore, that the cellular factors required to form a functional *trans*-activating complex with Vmw65 are present in neuronal cells. No *trans*-activation of IE-CAT was observed, however, when only a low DNA con-

Fig. 1. Assay of CAT activity following transfection of IE-CAT into C1300 cells (tracks 1-4) and permissive BHK cells (tracks 5-8)

Cells were transfected by the method of Gorman (1985) using either 5 µg (tracks 1, 2, 5 and 6) or 1 µg (tracks 3, 4, 7 and 8) of IE-CAT DNA, with (tracks 2, 4, 6 and 8) or without (tracks 1, 3, 5 and 7) 5 µg of pMCI DNA encoding Vmw65 (Campbell *et al.*, 1984).

centration was transfected, although the very low basal activity of the promoter was visible on long autoradiographic exposure of the chromatography plate. As in our previous experiments (Kemp *et al.*, 1990), the RSV promoter in the construct RSV-CAT directed a similar level of CAT activity in C1300 and BHK cells, confirming that differences in transfection efficiency are not responsible for the differences in IE-CAT activity in the two cell types (results not shown and Fig. 2d). Similar results were obtained using a Moloney murine leukaemia virus promoter to drive expression of Vmw65, indicating that the effects we observe are not due to differences in the expression of Vmw65 in the two cell types (results not shown).

To investigate these effects further, we studied the dependence of *trans*-activation on the concentration of Vmw65. As shown in Fig. 2, clear *trans*-activation was observed as before in C1300 cells with 5 µg of IE-CAT, and the *trans*-activation showed a dependence on the concentration of Vmw65 similar to that observed in BHK cells. As before, however, no *trans*-activation was observed with a low concentration of IE-CAT, regardless of the amount of Vmw65 added, even though the low basal activity of the IE promoter was detectable. As before, similar levels of RSV-CAT activity were observed in both cell types (Fig. 2d).

The ability of Vmw65 to *trans*-activate the IE promoter at high concentrations of IE-CAT suggested that the failure of *trans*-activation at low concentrations of IE-CAT might be dependent on the neuronal cell-specific TAATGARAT binding protein that we have previously characterized (Kemp *et al.*, 1990). At low concentrations of IE-CAT, this factor would interfere with the binding of the *trans*-activating complex to the TAATGARAT motif, but it would be titrated out at high IE-CAT concentrations, allowing *trans*-activation to occur. If this were the case, *trans*-

Fig. 2. Effect of different pMCI/IE-CAT molar ratios on the activity of IE-CAT

Activity was measured in C1300 cells using 1 µg (a) or 5 µg (b) of IE-CAT, and in BHK cells using 1 µg of IE-CAT (c). Panel (d) shows the activity obtained following transfection of 1 µg of an RSV-CAT construct into either C1300 or BHK cells.

Fig. 3. Effect of different pMCI/IE-CAT molar ratios on CAT activity in the presence of the TAATGARAT motif

The activity of 1 µg of IE-CAT transfected into C1300 cells in the presence of 4 µg of the pF plasmid containing a TAATGARAT motif was measured.

HSV IE consensus	Pu Pyr G N T A A T G A Pu A T
Octamer consensus	A T G C A A A T N A
U1 octamer: -219 to -210	A T G T A G A T G A
U3 octamer: -226 to -217	A T G C T A A T T A

Fig. 4. Comparison of the HSV IE control sequence TAATGARAT (Whitton & Clements, 1984) with the octamer consensus sequence (Falkner *et al.*, 1986) and the octamer motifs up-stream of the human U1 (Lund & Dahlberg, 1984) and U3 (Suh *et al.*, 1986) genes

The single base changes in the consensus sequence found in the U1 and U3 genes, rendering them respectively less or more like the viral sequence, are indicated.

activation would also be produced at low IE-CAT concentrations by including in the transfection a plasmid containing a cloned TAATGARAT motif capable of binding to the repressor. As shown in Fig. 3, this is indeed the case: co-transfection of 1 µg of IE-CAT with 4 µg of the TAATGARAT-containing plasmid pF resulting in *trans*-activation of the IE promoter by Vmw65 at a low IE-CAT concentration.

Both Oct-1 and the neuronal cell repressor protein that we have previously characterized (Kemp *et al.*, 1990) can also bind to the octamer motif which is present in many different cellular genes (for review, see Falkner *et al.*, 1986) and is related to TAATGARAT at the DNA sequence level (see Fig. 4). We have previously shown that binding of the Oct-1/Vmw65 complex to the octamer motif in cellular genes can result in *trans*-activation of some of these genes as well (Kemp & Latchman, 1988; Dent *et al.*, 1990). Interestingly, whether or not such binding and *trans*-activation occurs is dependent upon the precise sequence of the octamer motif in each cellular gene. Thus, following transfection of Vmw65 into BHK cells, the cellular gene encoding the small nuclear RNA U3, which has a TAATGARAT-like octamer (Suh *et al.*, 1986; see also Fig. 3), was up-regulated, whereas that encoding U1, which has an octamer motif very different from TAATGARAT (Lund & Dahlberg, 1984), was unaffected (Kemp & Latchman, 1988).

To determine whether these effects could also be observed in neuronal cells in the presence of the additional neuronal octamer binding protein, we transfected the plasmid pMCI (encoding Vmw65) into C1300 cells and measured the effect on transcription of the U1 and U3 genes using a nuclear run-on assay (Patel *et al.*, 1986) in conjunction with cloned U1 and U3 probes. In these experiments (Table 1) *trans*-activation of the U3 but not of the

Table 1. Effect of transfection of Vmw65 on transcription of the cellular U1 and U3 genes

Values are c.p.m. binding to the indicated clone (U1, U3, 123) in nuclear run-on assays carried out with nuclei prepared from cells transfected with either pAT 153 vector or plasmid pMCI (encoding Vmw65; Campbell *et al.*, 1984). The values given are the averages of two determinations whose range is given in brackets. '123' indicates the control clone derived from a cellular gene whose mRNA level is unaltered by HSV infection or transfection of Vmw65 (Kemp *et al.*, 1986).

Clone	C1300 cells		BHK cells	
	pAT	pMCI	pAT	pMCI
U1	63 (7)	65 (6)	51 (6)	54 (5)
U3	14 (2)	53 (4)	24 (2)	75 (6)
123	225 (17)	243 (19)	235 (19)	225 (21)

U1 gene was observed in C1300 cells, exactly as in BHK cells. Hence *trans*-activation of cellular genes by Vmw65 can be observed in non-permissive cells of neuronal origin, as well as in permissive cells, and exhibits the same sequence specificity with regard to the activation of cellular genes with different octamer motifs.

DISCUSSION

When a high concentration of the IE-CAT construct is transfected into C1300 cells with Vmw65, *trans*-activation of the IE promoter can readily be observed exactly as in permissive BHK cells. Similarly, *trans*-activation of the cellular U3 gene, but not of the U1 gene, is observed in both BHK and C1300 cells following transfection of Vmw65. Thus the cellular factors required for *trans*-activation of viral and cellular genes by Vmw65 clearly exist in C1300 cells in forms which are indistinguishable from those observed in permissive cells. Indeed, our recent finding that *trans*-activation of IE genes by Vmw65 also occurs in immortalized cell lines derived from dorsal root ganglion neurons (S. C. Wheatley & D. S. Latchman, unpublished work) suggests that these factors are present in most neuronal cells, and in particular in sensory neurons (the natural site for latent infection with HSV *in vivo*).

Interestingly, however, at a low concentration of IE-CAT, *trans*-activation by Vmw65 was not observed in C1300 cells, although it was still observed in BHK cells. This lack of *trans*-activation at a low concentration of the target promoter is dependent on the presence in neuronal cells of the cellular repressor protein which we have previously reported as binding to the TAATGARAT motif in the IE promoters (Kemp *et al.*, 1990). Thus, at a low concentration of the IE promoter, the target site for binding of the *trans*-activating complex of Vmw65 and Oct-1 will be occupied by the repressor, and *trans*-activation will not occur. At a higher concentration of IE-CAT or in the presence of the TAATGARAT-containing plasmid pF, the repressor will be titrated out, allowing binding and *trans*-activation by Vmw65 to occur.

It is clear, therefore, that failure of IE gene expression and of the lytic cycle in these cells is not due to a lack of formation of the *trans*-activating complex. Rather, the primary cause of this failure is likely to be the binding of a neuronal-cell-specific octamer/TAATGARAT-binding protein to the TAATGARAT motifs in the IE promoters. At the low level of virus which reaches a sensory ganglion cell, this factor will occupy the TAATGARAT sites in these promoters, preventing binding of

Oct-1 and of the Oct-1/Vmw65 complex, and hence inhibiting both the basal activity of the IE promoters and their *trans*-activation. At high levels of virus, such as occurs during re-activation of latent HSV by super-infection of latently infected neurons *in vitro* (Wilcox & Johnson, 1988), the low levels of the neuronal protein will be titrated out by the excess TAATGARAT elements, resulting in increased basal activity and *trans*-activation of the IE promoters. Although the neuronal factor is also likely to play a role in cellular gene regulation in neuronal cells, it does not appear to prevent basal expression or activation of the constitutively expressed U1 and U3 genes in C1300 cells. Interestingly, recent studies (C. L. Dent & D. S. Latchman, unpublished work) suggest that binding of the repressor is strongly affected by the precise sequence of the octamer/TAATGARAT motif and by flanking sequences. Hence its failure to inhibit either U1 or U3 gene expression may be explained by a failure to bind to the octamer motifs in these genes. Further studies are required on the role of the neuronal factor in cellular gene regulation as well as its modulation in response to stimuli which cause the re-activation of latent virus *in vivo*.

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