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Transcriptional Regulation of the VP16 Gene of Herpes Simplex Virus Type 1

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The promoter of the HSV-1 VP16 gene contains binding sites for the cellular transcription factors such as USF, CTF, and Sp1, each of which affects basal level expression of the VP16 gene. Transcription of the VP16 gene was induced by viral immediate-early proteins, ICP0 and ICP4, in a synergistic manner but repressed by ICP22. To gain further insight into the role of ICP0 in the expression of the VP16 gene during virus infection, several mutants with deletions in each of their transcriptional regulatory elements were generated. According to transient gene expression assays of these mutants using the CAT gene as a reporter, the USF and CTF binding sites were necessary for efficient induction of the promoter in the presence of transfected ICP0 or during virus infection, whereas the Sp1 binding site had little effect on ICP0-mediated VP16 expression. These results indicate that the immediate early proteins of HSV-1 regulate expression of the VP16 gene during virus infection by modulating the activities of cellular transcription factors such as USF and CTF.

Keywords: CTF, HSV, ICP0, USF, VP16.

Introduction

Expression of herpes simplex virus type 1 (HSV-1) genes is coordinately and sequentially controlled, such that three major phases of protein synthesis occur (Honess and Roizman, 1974; 1975). The immediate-early or alpha genes of herpes simplex virus type 1 (HSV-1) are the first viral genes expressed during the infectious cycle. These genes are expressed in the absence of prior viral protein synthesis and specify five proteins designated infected-cell polypeptides (ICP) including ICP0, ICP4, ICP22, ICP27, and ICP47 (Clements *et al.*, 1977). All of these immediate-early proteins, except ICP47, are involved in regulating immediate-early, early, or late gene expression (Sekulovich *et al.*, 1988; McCarthy *et al.*, 1989; Su and Knipe, 1989). In particular, ICP4 is a major regulatory protein that modulates viral gene expression during virus replication (DeLuca *et al.*, 1985; DeLuca and Schaffer, 1988). ICP0, on the other hand, is not essential with regard to virus replication (Stow and Stow, 1986; Sacks *et al.*, 1987). However, the existence of a functional ICP0 provides a selective advantage for virus growth in tissue culture (Cai and Schaffer, 1989; Chen and Silverstein, 1992; Cai *et al.*, 1993). Studies have demonstrated that ICP0 functions as a regulatory protein activating the expression of all three classes of viral genes and a number of heterologous promoters either by itself or in a synergistic mode with ICP4 (Cai and Schaffer, 1992; Jordan and Schaffer, 1997; Samaniego *et al.*, 1997; Davido and Leib, 1998).

The virion protein VP16 (Vmw65, α-TIF, ICP25) of HSV performs two known functions during the virus life cycle. It is a major structural component of the virion (Spear and Roizman, 1972; Heine *et al.*, 1974) and as such has an essential function for normal virus assembly (Ace *et al.*, 1988; Moss, 1989). VP16 also specifically transactivates immediate-early (IE) gene expression after virus infection (Campbell *et al.*, 1984). This function is dispensable for infection in tissue cultures at high multiplicity but is essential for normal virus replication at low multiplicities of infection and for virulence after intracranial or intraperitoneal inoculation in mice (Ace *et al.*, 1989).

A comparison of upstream sequences of the VP16 genes of HSV-1 and 2 revealed good conservation of proximal promoter elements (Greaves and O'Hare, 1991). Both promoters contain several binding sites for gene regulatory proteins such as USF, CTF, and Sp1. Expression of the HSV-2 VP16 gene is induced by either ICP0 or ICP4 (Greaves and O'Hare, 1991). Mutational analysis of the HSV-2 promoter demonstrated that the integrity of Sp1 and USF binding sites is important for the full activity of the

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promoter. However, the ratio of induction by either ICP0 or ICP4 protein was approximately the same for both wild-type and mutant promoters, suggesting that USF and Sp1 are not required for the mediation of induction by viral IE products. Although the VP16 gene of HSV-1 showed a high degree of sequence conservation both in regulatory and coding regions relative to the HSV-2 gene, the expression mechanism of the HSV-1 gene is not understood. The aim of this study was to investigate the expression mechanism of the HSV-1 VP16 gene during virus infection, and to compare it with that of the HSV-2 VP16 gene.

Materials and Methods

Growth and transfection of animal cells BHK-21 cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. Cells were seeded at 3×10^5 cells/6 cm dish and transfected the next day with a calcium phosphate-DNA precipitate containing 3 μg each of target and effector plasmid DNAs, as previously described (Gorman, 1985). The level of expression from the target gene (CAT activity) was analyzed at 40 h after transfection. When appropriate, cells were infected at 28 h post-transfection with 5 PFU of HSV-1 KOS per cell and harvested 12 h later. Each experiment was repeated at least two times, and although there were variations in the absolute CAT activity from one experiment to the next, the profile of the response was always consistent.

Plasmid construction VP16(−237/+158) CAT, the parent construct for the promoter studies, contains a 395-bp *Xho*I-*Eco*RV fragment from the VP16 promoter of HSV-1 strain KOS in front of the CAT structural gene. VP16(−85/+158) CAT, VP16(−73/+158) CAT, VP16(−60/+158) CAT, and VP16(−48/+158) CAT were constructed by PCR-mediated subcloning of the parent plasmid.

CAT assays Cell lysates were analyzed for CAT activity at 40 h after transfection according to the technique of Gorman (1985). A quantitative estimate of chloramphenicol acetylation was obtained by excision of the substrate and products from thin-layer chromatography plates, and subsequent measurement by liquid scintillation counting in an LKB 216 scintillation counter. The data are presented as n-fold increase over control.

Results

Initial studies were carried out to characterize the HSV-1 VP16 promoter with respect to its response to virally-encoded *trans* activators in transfection assays. Plasmid VP16 (−237/+158) CAT, which contains a fragment of the upstream region of the VP16 gene fused to the structural gene for CAT (Fig. 2A), was cotransfected into BHK-21 cells together with an equal quantity of pUC18 as a negative control, or with a plasmid encoding either ICP0 (Everett, 1986), ICP4 (Everett, 1986), ICP22 (Everett, 1986), ICP27 (Rice and Knipe, 1990), or ICP47 (Umene, 1986). The results of one representative series of assays are shown in Fig. 1 and summarized in Table 1. CAT activity was greatly induced by cotransfection with a plasmid expressing ICP0 (Fig. 1, lane 2). Also, ICP4 and ICP27 could induce expression of the HSV-1 promoter (Fig. 1, lanes 3 and 4, respectively), although the activation efficacy was much lower compared to that of ICP0. However, cotransfection of other IE genes did not induce the VP16 promoter and, when ICP22 was involved, the expression level was slightly lower than the basal level (Fig. 1, lane 5).

Next, we examined whether the combination of IE products could result in synergistic activation of the VP16 promoter. As ICP0 was the most potent activator of the VP16 promoter, ICP0-expressing plasmid was transfected in combination with a plasmid encoding ICP4, ICP22, ICP27, or ICP47. As summarized in Table 1, the activity obtained from VP16 (−237/+158) CAT was very high when ICP0 and ICP4 were transfected in combination, suggesting the synergistic activation of the VP16 promoter

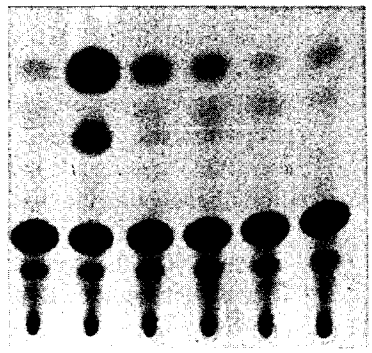

Fig. 1. Transactivation of the VP16 CAT construct by cotransfection with IE expression vectors. BHK cells were transfected with 3 μg of VP16(−237/+158) CAT and 3 μg each of pUC18 (track 1) or a plasmid encoding either ICP0 (track 2), ICP4 (track 3), ICP27 (track 4), ICP22 (track 5), or ICP47 (track 6). CAT activity in soluble extracts was measured at 48 h post-transfection.

Table 1. Regulation of the VP16 promoter by HSV immediate-early proteins.

Effector	Fold increase of CAT activity
pUC18	1.0
ICP0	15.5
ICP4	3.4
ICP22	0.4
ICP27	2.1
ICP47	1.1
ICP0+ICP4	28.5
ICP0+ICP22	0.8
ICP0+ICP27	18.2
ICP0+ICP47	7.2

by these two IE gene products. On the other hand, addition of ICP22 resulted in lower levels of CAT activity, suggesting that this IE product might act as a negative regulator of VP16 expression during the early stages of virus infection. Interestingly, when ICP47 was transfected in combination with ICP0, it reduced the original ICPO increase of CAT activity from 15.5-fold to 7.2-fold, whereas ICP47 alone did not affect the VP16 promoter. We do not understand how this phenomenon occurred or whether this situation also occurs during virus infection.

To define critical *cis*-acting elements within the −237 to +158 region responsible for the induction of the VP16 promoter during virus infection, four deletion mutants were constructed with stepwise deletions of the transcriptional regulatory elements (Fig. 2A). These results of the CAT assay are shown in Fig. 2B and summarized in Table 2. When the reporter with a deletion between −237 and −86 was tested, the increase of CAT activity after treatment with either ICP0 or HSV-1 was not reduced (Fig. 2B, lanes 5 and 6, respectively). Similarly, although the basal level of CAT activity was reduced to 55% of the parent VP16 promoter when the first USF binding site was deleted (Fig. 2, lane 7), activation either by transfected ICP0 or HSV-1 superinfection was still increased if the reduced level of basal activity was considered (Fig. 2B, lanes 8 and 9, respectively). These results indicate that the sequence upstream of −73, including the first USF binding site, is not required for activation of the VP16 promoter by ICP0 during virus infection. On the contrary, a deletion that extended to −60, removing both the first USF binding site and a CTF binding site, reduced the effect of ICP0 on the expression of the VP16 gene to significantly lower levels (Fig. 2, lane 11). However, the mutated promoter could be sufficiently activated by HSV-1 infection (Fig. 2, lane 12) or by the combination of ICP0 and ICP4 (Table 2), suggesting that other IE products or combinations of IE products could still activate the VP16 promoter in the absence of CTF binding.

Table 2. Mutation analysis of the VP16 promoter.

Reporter plasmid	Basal activity (%)[a]	Fold increase of CAT activity after treatment with[b]		
		ICP0	ICP0+ICP4	HSV-1
VP16(−237/+158) CAT	100	8.5	25.3	35.2
VP16(−85/+158) CAT	70	11.0	32.5	42.3
VP16(−73/+158) CAT	55	13.2	30.8	56.4
VP16(−60/+158) CAT	58	3.6	28.5	32.5
VP16(−48/+158) CAT	40	1.2	1.8	2.2

[a] The activity of each CAT construct, when cotransfected with a negative control, pUC18, was compared to that of VP16(−237/+158) CAT which contains the entire VP16 promoter.
[b] The data are presented as n-fold increase over control.

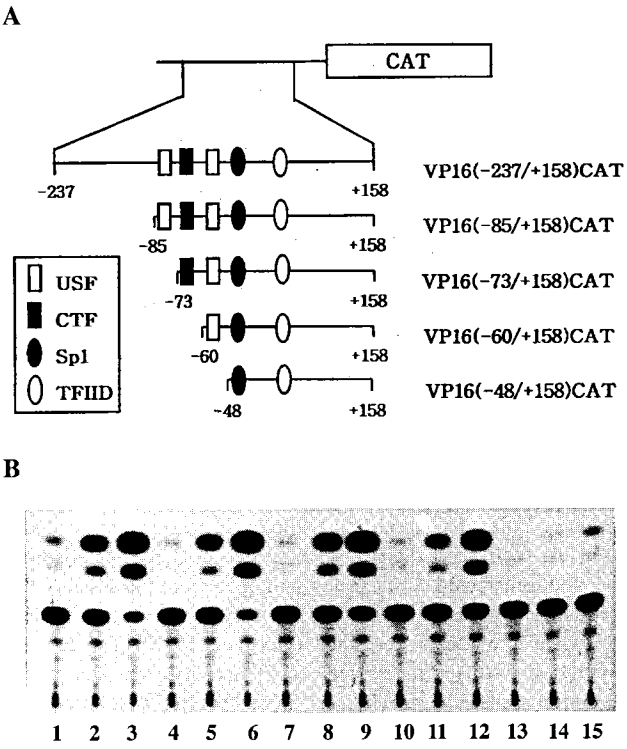

Fig. 2. Mutation analysis of the VP16 promoter. **A.** Diagrammatic representation of the VP16-CAT constructs used. Transcription factors binding to each site are indicated in the box. **B.** CAT activities of the constructs. VP16(−237/+158) CAT (tracks 1 to 3), VP16(−85/+158) CAT (tracks 4 to 6), VP16(−73/+158) CAT (tracks 7 to 9), VP16(−60/+158) CAT (tracks 10 to 12), or VP16(−48/+158) CAT (tracks 13 to 15) were cotransfected with pUC18 (tracks 1, 4, 7, 10, and 13) or ICP0 (tracks 2, 5, 8, 11, and 14), respectively, and their activities were measured. For tracks 3, 6, 9, 12, and 15, cells were transfected with the corresponding reporter plasmid and then infected with HSV-1 at a multiplicity of 5 PFU.

Furthermore, when the deletion was extended to −48, removing both USF binding sites and a CTF binding site, ICP0 transactivation was reduced to almost background levels (Fig. 2, lane 14), indicating that the second USF binding site is also important for activation of the VP16 promoter by ICP0. Also, this site seems to be critical for activation of the VP16 gene during virus infection (Fig. 2, lane 15) and for the synergistic effect of ICP0 and ICP4 (Table 2). Even with this mutant, promoter activity was slightly induced by HSV-1 superinfection. This may be explained by considering that other IE gene products such as ICP4 and ICP27 could transactivate the mutant promoter during virus infection.

Discussion

Although ICP0 may most strongly contribute to expression of the VP16 gene, our data in the present study suggest that several combinations of regulatory proteins might

participate in the expression of the VP16 gene at various stages of virus infection. This is particularly true when all regulatory proteins of HSV-1 coexist during the immediate-early stage of infection. Under this situation, in spite of plentiful positive factors such as ICP0 and ICP4, the expression of the VP16 gene could still be prohibited because the strong negative regulator, ICP22, is also present.

Although two transcription factor binding sites seem to be important for activation of the VP16 gene by ICP0, the USF site might be more important for the expression of the gene during viral infection, as other regulatory proteins of HSV-1 such as ICP4 could complement transcription of the VP16 gene in the absence of CTF binding to the promoter. However, for the VP16 gene of HSV-2, USF binding to the HSV-2 promoter is known to contribute to the high basal activity of the promoter but is not required for the mediation of induction by viral IE products (Greaves and O'Hare, 1991). Why the two homologous promoters respond differently to the IE proteins remains to be investigated.

According to our previous results (Kwun *et al.*, 1999), ICP0 transactivated the TK promoter and the induced activity completely disappeared when the CTF binding site was mutated. Our present studies also show that CTF in combination with USF is involved in the transactivation of the VP16 gene by ICP0, suggesting that ICP0 might regulate the expression of viral genes by changing the activities of cellular TFs.

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