The herpes simplex virus virion protein Vmw65 transcriptionally activates the gene encoding the U4 snRNA but not that encoding the U2 snRNA during lytic infection

David S. LATCHMAN

Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, U.K.

Although lytic infection with herpes simplex virus (HSV) causes the repression of most host cell biosynthesis, it results in increased transcription of the cellular gene encoding the U4 snRNA, leading to accumulation of this snRNA. In contrast, no increased transcription of the gene encoding the U2 snRNA or accumulation of this RNA is observed in infected cells. These effects are mediated by the HSV virion protein Vmw65, which activates the U4 gene but does not affect the U2 gene. The significance of this difference between the U2 and U4 genes is discussed with regard to the presence in both of these genes of an identical octamer-binding site for the cellular transcription factor Oct-1 which complexes with Vmw65.

INTRODUCTION

Lytic infection of cells with herpes simplex virus (HSV) produces a decline in the synthesis of most cellular RNAs and proteins (reviewed by Fenwick, 1984). This repression is produced by a number of different mechanisms including decreased rates of gene transcription (Stenberg & Pizer, 1982; Kemp & Latchman, 1988a). Some cellular proteins such as the histones are unaffected by this repression however (Mayman & Nishioka, 1985), whereas others such as the 90 kDa heat-shock protein (La Thangue & Latchman, 1988), the Sm auto-antigen (Bachmann *et al.*, 1986; Sharpe *et al.*, 1989) and ubiquitin (Latchman *et al.*, 1987) actually increase in abundance during lytic infection. In several such cases, these increases have been shown to reflect transcriptional induction of the corresponding genes (Patel *et al.*, 1986; Latchman *et al.*, 1987).

These examples are of interest in that they are likely to reflect the interaction of viral activator proteins with cellular transcription factors and regulatory mechanisms. Thus we have previously shown (Kemp & Latchman, 1988b) that the cellular gene encoding the U3 snRNA is transcriptionally induced during HSV infection, whereas that encoding the U1 snRNA is not. The promoters of both of these genes contain the octamer motif (ATGCAAAT) which binds the cellular transcription factor Oct-1 (Lund & Dahlberg, 1984; Suh *et al.*, 1986; for review see Falkner *et al.*, 1986). During lytic infection, the HSV virion protein Vmw65 forms a complex with Oct-1 which binds to the octamer-related TAATGARAT motif in the viral immediate-early gene promoters (Fig. 1) and induces their expression (Gerster & Roeder, 1988; O'Hare & Goding, 1988; Preston *et al.*, 1988). The differential induction of the U1 and U3 snRNA genes in lytic infection is dependent upon differences in the octamer sequence in their respective promoters (Kemp & Latchman, 1988b). Thus the U3 gene has an octamer motif which differs from the consensus octamer in a manner which makes it more like the TAATGARAT motif (Suh *et al.*, 1986; see Fig. 1 in present paper) and is hence transcriptionally activated by Vmw65. Conversely, the U1 octamer is less like TAATGARAT than the consensus octamer (Lund & Dahlberg 1984; see Fig. 1) and is unaffected by Vmw65.

Here I report the effect of HSV infection and of Vmw65 on the genes encoding two other snRNAs, U2 and U4, both of which contain octamer motifs conforming exactly to the consensus (Weston *et al.*, 1984; Bark *et al.*, 1986; Mangin *et al.*, 1986).

MATERIALS AND METHODS

Cells and viruses

BHK-21 cells (clone 13: Macpherson & Stoker, 1962) were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum. Cells were infected with 5 plaque-forming units of HSV-1 strain F (Ejercito *et al.*, 1968) and were harvested 6 h after infection for the isolation of RNA and nuclei. Infections in the presence of cycloheximide were carried out using a concentration of 0.2 mg of the drug/ml, which was added 1 h before infection. In all cases, mock-infected cells were similarly treated without addition of virus.

Isolation of RNA and Northern blotting

RNA was isolated from mock-infected and infected cells by the guanidinium isothiocyanate procedure as described by

Octamer consensus	A T G C A A A T N A
HSV immediate-early consensus	R Y G C T A A T G A R A T
U1	A T G T A G A T G A G G C
U3	A T G C T A A T T A A G C
U2	A T G C A A A T T C G A A
U4	A T G C A A A T C A A C G

Fig. 1. Comparison of the consensus octamer motif (Falkner *et al.*, 1986), the viral TAATGARAT consensus (Whitton & Clements, 1984) and the octamer motifs in the human U1 (Lund & Dahlberg, 1984), U2 (Mangin *et al.*, 1986), U3 (Suh *et al.*, 1986) and U4 (Bark *et al.*, 1986) snRNA genes

The single sequence changes from the consensus octamer which render the U1 and U3 octamer motifs less or more like the TAATGARAT element are indicated (v). For comparison, the sequences adjacent to the octamer in the U snRNA genes which correspond in position to the GARAT portion of the viral sequence are also shown.

Chirgwin *et al.* (1979). For Northern blotting of the U2 and U4 RNAs, which are less than 200 bp in size, total RNA was run on a 7 M-urea/12.5% (w/v) acrylamide gel, electroblotted on to Biotodyne nitrocellulose membranes (Bio-Rad) as previously described (Kemp & Latchman, 1988b) and hybridized with probes specific for the U2 and U4 snRNAs (Weston *et al.*, 1984; Bark *et al.*, 1986). For hybridization with the control 123 probe, which detects an RNA whose level does not alter in HSV infection (Kemp *et al.*, 1986), samples were run on a conventional formaldehyde/agarose gel and transferred to nitrocellulose as described by Murphy *et al.* (1983).

Nuclear run-on assay

Nuclei were isolated as described by Patel *et al.* (1986) 6 h after mock-infection or infection with HSV-1 and were allowed to continue transcription for 1 h under run-on conditions [50 mM-Hepes, pH 7.9, 120 mM-KCl, 20 mM-dithiothreitol, 30 mM- β -mercaptoethanol, 5 mM-magnesium acetate and 2 mM-MnCl₂ in the presence of 50 μ Ci of ³²P-labelled GTP (3000 Ci/mmol)]. Labelled transcripts isolated from the nuclei were then used to probe a dot-blot on to which 2 μ g of each of the appropriate plasmid DNAs had been spotted. After hybridization and washing, filters were autoradiographed and labelled spots were cut out and counted for radioactivity.

Transfection

Cells were transfected with either the plasmid pMCI (encoding Vmw65; Campbell *et al.*, 1984) or the pAT153 vector as described by Gorman (1985), except that the calcium phosphate precipitate was left on the cells for 16 h and the glycerol shock was omitted. A 10 μ g portion of the appropriate DNA was added to 2×10^6 cells on a 90 mm plate.

RESULTS AND DISCUSSION

To test the effect of HSV infection on the U2 and U4 snRNA genes, BHK-21 cells (Macpherson & Stoker, 1962) were infected with HSV-1 strain F (Ejercito *et al.*, 1968) or mock-infected, and RNA was prepared 6 h after infection. Northern blotting of this RNA with U2- and U4-specific probes revealed an increased abundance of the U4 RNA in the infected cells, whereas no increase was observed in the U2 RNA (Figs. 2a and 2b). No difference between the uninfected and infected samples was

observed in the level of the specific RNA recognized by the control clone, which is known not to change in abundance during HSV infection (Kemp *et al.*, 1986), confirming that equal amounts of RNA had been loaded in each track (Fig. 2c).

To determine whether the change in the level of the U4 RNA was mediated by increased transcription of the corresponding gene, I carried out a nuclear run-on assay using nuclei prepared from uninfected and infected cells. As shown in Table 1, increased transcription of the U4 gene was readily observed in the infected cells, although the level of U2 transcription remained unchanged, as did that of the control tRNA clone, in accordance with our previous observation (Jang & Latchman, 1989).

Having established that the U4 gene is transcriptionally induced in HSV infection, I investigated the stage of infection at which this occurs. To do this, cells were infected in the presence of the protein synthesis inhibitor cycloheximide (0.2 mg/ml). Under these conditions, the virion carrying Vmw65 will enter the cell and transcription of the viral immediate-early genes will be induced. The transcripts from these genes will not be translated into protein, however, due to the presence of the drug. Hence the lytic cycle which is dependent on the viral immediate-early proteins will be aborted at an early stage (Watson & Clements, 1980). The rate of transcription of the U4 gene was therefore compared in nuclei isolated from cells infected in the presence of cycloheximide and nuclei from cells which had been mock-infected in the presence of a similar concentration of cycloheximide. As shown in Table 2, increased transcription of the U4 gene was still observed in the infected cells under these conditions, although transcription of the U2 gene and the control clone was unaffected by infection under these conditions.

The transcriptional induction of the U4 gene by HSV can therefore occur without viral protein synthesis in the infected cells and is dependent upon some early event in viral infection, such as viral binding to the cell surface or cellular entry of the virion. I therefore investigated whether this effect was dependent upon the entry of the transcriptional activator protein Vmw65 in the incoming virion. To do this, cells were transfected with a plasmid (pMCI) containing the gene encoding Vmw65 (Campbell *et al.*, 1984). The rate of transcription of the endogenous U4 gene was enhanced in the transfected cells compared with that observed in cells transfected with plasmid vector alone (Table 3), whereas the U2 gene was, as before, unaffected. In contrast, no effect on U2 or U4 was observed following transfection of the gene encoding another HSV transcriptional activator, Vmw175 (ICP4), indicating that this effect was specific to Vmw65.

Hence the U4 snRNA gene, like that encoding the U3 snRNA, is transcriptionally induced in HSV infection by the virion protein Vmw65. Moreover, this induction is sufficiently strong to be observable in a transfection experiment where only a minority of cells take up the transfected DNA. In contrast, the U2 gene, like that encoding U1, is unaffected by Vmw65 in transfection or infection. Interestingly, HSV infection also leads to increased accumulation of the Sm proteins (Bachmann *et al.*, 1986; Sharpe *et al.*, 1989) which are associated with the U snRNAs (except U3) in the spliceosome (Maniatis & Reed 1987). Such increased accumulation of some components of the mRNA-splicing apparatus in infected cells is unlikely to be of benefit to the virus, since relatively few HSV RNAs are spliced. However, in view of the potential role of Sm proteins in polyadenylation (Moore & Sharpe, 1985), it is possible that accumulation of these proteins and their associated snRNAs may play a role in the change in usage of alternative polyadenylation sites which has been observed in infected cells (McLauchlan *et al.*, 1989).

As with the U3 snRNA gene, the induction of the U4 gene by Vmw65 is likely to be dependent upon the octamer motif within its promoter, the consensus octamer motif in the histone H2B

Fig. 2. Northern blot of RNA from mock infected (M) or infected (I) cells probed with U2 (a) and U4 (b) genomic clones or with a cDNA clone (123) of an mRNA whose level does not change in HSV infection (c; Kemp *et al.*, 1986).

Panes (a) and (b) were obtained by electrophoresis on a denaturing acrylamide gel and panel (c) by electrophoresis on a denaturing agarose gel, as described in the Materials and methods section. Arrowheads indicate the predicted sizes of the U2 and U4 RNAs and the positions of the 28 S and 18 S ribosomal RNA markers.

Table 1. Effect of HSV infection on transcription of the U2 and U4 genes

Values (averages of three determinations; ranges given in parentheses) are c.p.m. binding to the indicated clone in a nuclear run-on assay after subtraction of background counts bound by the pUC8 vector.

Clone	Cells ...	Radioactivity bound (c.p.m.)	
		Mock-infected	Infected
U2		31 (7)	33 (6)
U4		37 (5)	99 (11)
tRNA		51 (6)	50 (8)

Table 2. Effects of HSV infection in the presence of cycloheximide on transcription of the U2 and U4 genes

Values (averages of two determinations; ranges given in parentheses) are c.p.m. binding to the indicated clone in a nuclear run-on assay after subtraction of the background counts bound by the pUC 8 vector (approx. 10 c.p.m.). Nuclei were prepared from cells which had been either mock-infected or infected in the presence of 0.2 mg of cycloheximide/ml.

Clone	Cells ...	Radioactivity bound (c.p.m.)	
		Mock-infected	Infected
U2		25 (3)	24 (2)
U4		33 (6)	101 (8)
tRNA		47 (5)	43 (5)

Table 3. Effect of transfecting Vmw65 or Vmw175 on transcription of the endogenous U2 and U4 genes

Values (averages of two determinations; ranges given in parentheses) are c.p.m. binding to the indicated clone in a nuclear run-on assay. Nuclei were prepared from 2×10^6 cells transfected with 10 μ g of the indicated DNA.

Clone	Promoter ...	Radioactivity bound (c.p.m.)		
		pAT	pMCI (Vmw65)	p175 (Vmw175)
U2		20	18 (1)	19 (2)
U4		29 (4)	63 (5)	27 (2)
tRNA		51 (5)	50 (4)	43 (3)

gene promoter having also been shown to render it inducible by Vmw65 (Latchman *et al.*, 1989). This raises the question of why the U2 gene is unaffected by Vmw65, even though, unlike the U1 gene, it has a consensus octamer motif. It is unlikely that this effect is due to differences between U4 and U2 in the region corresponding to the GARAT portion of the TAATGARAT motif, since the U4 gene sequence matches this sequence at only one position, compared with two for the U2 gene sequence (see Fig. 1). Interestingly, however, the octamer motif in the U2 gene

is located adjacent to a binding site for the transcription factor Sp1 (Dynan & Tjian, 1983) which is necessary for U2 gene expression (Ares *et al.*, 1987) and which is absent in the U4 gene. Similarly the octamer motif, as conventionally written, is orientated away from the transcribed region of the U4 gene (Bark *et al.*, 1986) and the H2B gene (Zhong *et al.*, 1983). It is possible, therefore, that binding of Sp1 to its site in the U2 promoter may interfere with binding of the Oct-1/Vmw65 transactivating complex. Alternatively, when binding to a consensus octamer this complex may need to bind in a particular orientation to achieve the appropriate interactions with other factors necessary to activate transcription. Further studies investigating the inducibility by Vmw65 of constructs containing the isolated U2 octamer in both orientations with and without the adjacent Sp1 site will be necessary to resolve these possibilities.

Whatever the case, however, it is clear that as well as repressing most host cell biosynthesis, HSV can induce increased transcription of some but not all snRNA genes, this effect being dependent on the interaction of the virion protein Vmw65 with the different sequences and contexts of the octamer motifs in each gene.

I thank John Estridge for excellent technical assistance and the following for kindly donating the plasmid clones: Roger Everett (p175), Ulf Pettersson (U2 and U4) and Chris Preston (pMCI). This work was supported by the Cancer Research Campaign.

REFERENCES

- Ares, M., Jr., Chung, J.-S., Giglio, L. & Weiner, A. M. (1987) *Genes Dev.* **1**, 808–817.
- Bachmann, M., Falke, D., Preuhs, J., Schroder, H. C., Pfeifer, K. & Muller, W. E. G. (1986) *J. Gen. Virol.* **67**, 2587–2594.
- Bark, C., Weller, P., Zabielski, J. & Pettersson, U. (1986) *Gene* **50**, 333–344.
- Campbell, M. E. M., Palfreyman, J. W. & Preston, C. M. (1984) *J. Mol. Biol.* **180**, 1–19.
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. I. (1979) *Biochemistry* **18**, 5294–5299.
- Dynan, W. S. & Tjian, R. (1983) *Cell* **35**, 79–87.
- Ejercito, P. M., Kieff, E. D. & Roizman, B. (1968) *J. Gen. Virol.* **2**, 357–364.
- Falkner, F. G., Moickat, R. & Zachau, H. G. (1986) *Nucleic Acids Res.* **13**, 7847–7863.
- Fenwick, M. L. (1984) *Compr. Virol.* **19**, 359–390.
- Gerster, T. & Roeder, R. G. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6347–6351.
- Gorman, C. M. (1985) in *DNA Cloning* (Glover, D. M., ed.), vol. 2, pp. 143–190. IRL Press, Oxford.
- Jang, K. L. & Latchman, D. S. (1989) *FEBS Lett.* **258**, 255–258.
- Kemp, L. M. & Latchman, D. S. (1988a) *Eur. J. Biochem.* **174**, 443–449.
- Kemp, L. M. & Latchman, D. S. (1988b) *EMBO J.* **7**, 4239–4244.
- Kemp, L. M., Brickell, P. M., La Thangue, N. B. & Latchman, D. S. (1986) *Biosci. Rep.* **6**, 945–951.
- Latchman, D. S., Estridge, J. K. & Kemp, L. M. (1987) *Nucleic Acids Res.* **15**, 7283–7293.
- Latchman, D. S., Partridge, J. F., Estridge, J. K. & Kemp, L. M. (1989) *Nucleic Acids Res.* **17**, 8533–8542.
- La Thangue, N. B. & Latchman, D. S. (1988) *Exp. Cell Res.* **178**, 169–179.
- Lund, E. & Dahlberg, J. E. (1984) *J. Biol. Chem.* **259**, 2013–2021.
- Macpherson, I. & Stoker, M. (1962) *Virology* **16**, 147–151.
- McLauchlan, J., Simpson, S. & Clements, J. B. (1989) *Cell* **59**, 1093–1105.
- Mangin, M., Ares, M. & Weiner, A. M. (1986) *EMBO J.* **5**, 987–995.
- Maniatis, T. & Reed, R. (1987) *Nature (London)* **325**, 673–678.
- Mayman, B. & Nishioka, Y. (1985) *J. Virol.* **53**, 1–6.
- Moore, C. L. & Sharpe, P. A. (1985) *Cell* **41**, 8844–8855.
- Murphy, D., Brickell, P. M., Latchman, D. S., Willison, K. & Rigby, P. W. J. (1983) *Cell* **35**, 865–871.

- O'Hare, P. & Goding, C. R. (1988) *Cell* **52**, 435–445
- Patel, R., Chan, W. L., Kemp, L. M., La Thangue, N. B. & Latchman, D. S. (1986) *Nucleic Acids Res.* **14**, 5629–5640
- Preston, C. M., Frame, M. C. & Campbell, M. E. M. (1988) *Cell* **52**, 425–434
- Sharpe, N. G., Isenberg, D., Williams, D. G. & Latchman, D. S. (1989) *J. Autoimmun.* **2**, 701–708
- Stenberg, R. & Pizer, L. I. (1982) *J. Virol.* **42**, 474–487
- Suh, D., Busch, J. & Reddy, R. (1986) *Biochem. Biophys. Res. Commun.* **137**, 1133–1140
- Watson, R. J. & Clements, J. B. (1980) *Nature (London)* **285**, 329–330
- Weston, G., Lund, E., Murphy, J. T., Peterson, U. & Dahlberg, J. E. (1984) *EMBO J.* **3**, 3295–3301
- Whitton, J. L. & Clements, J. B. (1984) *Nucleic Acids Res.* **12**, 2061–2079
- Zhong, R., Heintz, N. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 7409–7425

Received 10 September 1990/23 November 1990; accepted 27 November 1990