Activation of the herpes simplex virus immediate-early gene promoters by neuronally expressed POU family transcription factors

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Herpes simplex virus immediate-early (IE) promoters contain the TAATGARAT motif which acts as a target site for the cellular POU family transcription factors Oct-1 and Oct-2. Here we show that other members of the POU family that are expressed in sensory neurons can also affect IE promoter activity. In particular, two members of the Brn-3 family of POU proteins Brn-3a and Brn-3c can activate the IE-1 and IE-3 promoters when co-transfected into fibroblasts and neuronal cells whereas

a third member Brn-3b represses IE promoter activity. In contrast, Brn-3 proteins cannot overcome the inhibitory effect of neuronal Oct-2 on IE promoter activity in co-transfections nor do they prevent transactivation of the IE promoters by the Oct-1–Vmw65 complex. The potential regulation of the IE promoters by several different neuronally expressed POU proteins during the initiation, maintenance and re-activation of latent infection in sensory neurons is discussed.

INTRODUCTION

Herpes simplex virus (HSV) establishes life-long latent infections of sensory neurons from which the virus can re-activate to allow repeated cycles of productive infection elsewhere in the body (for reviews see refs. [1,2]). It has been shown that latently infected cells do not express the viral immediate-early (IE) RNAs and proteins [3,4]. Similarly latent infections can be established by viral mutants that do not express individual IE genes or the virion transactivator Vmw65 [5,6] as well as by wild-type virus under conditions in which no IE gene expression or viral DNA replication has taken place [7,8].

These findings have led to the idea that the establishment and maintenance of latent infection is likely to involve a failure of IE gene expression hence preventing the lytic cycle from occurring. In normal lytic infection, such IE gene expression is dependent on the formation of a complex between the HSV protein Vmw65 and the cellular octamer-binding transcription factor Oct-1 which binds to the octamer-related TAATGARAT motif in the IE promoters [9,10]. We have previously shown that both neuronal cell lines and primary sensory neurons express an additional octamer-binding protein Oct-2 [11] which is capable of binding to the TAATGARAT motif and inhibiting both the basal activity of the IE promoter [12] and its transactivation by Vmw65 [13]. Hence this factor may play a critical role in inhibiting IE gene expression after the initial infection of neuronal cells, thereby resulting in the onset of latent infection.

Although considerable attention has therefore been devoted to elucidating the initial stages of latent infection, little attention has been paid to the transcriptional mechanisms mediating re-activation. However, it has been shown that the IE genes are expressed during re-activation *in vivo* from neuronal cells either latently infected *in vivo* and then explanted [14] or similarly infected directly *in vitro* and then exposed to re-activation stimuli [15]. Hence suitable stimuli must be able to induce viral IE gene transcription in neuronal cells.

Although the critical role of the TAATGARAT motif in controlling IE gene transcription during both lytic infection and the onset of latent infection suggests that it may also be involved

in the latter process, this effect is unlikely to be dependent on the activation of this motif via the Oct-1–Vmw65 complex, as occurs in lytic infection. Thus the virion transactivator Vmw65 would not be present in latently infected neurons as it is normally produced late in the viral lytic cycle after the prior expression of the IE genes [16]. Similarly the Oct-1 protein is a weak transactivator in the absence of Vmw65 and would have a minimal stimulatory effect on the IE promoter when Vmw65 is absent [17].

The lack of effect of Oct-1 in the absence of Vmw65 and the inhibitory effect of Oct-2 suggest that other neuronally expressed members of the POU family of transcription factors (to which Oct-1 and Oct-2 belong [18]) might be able to stimulate viral IE gene expression and thereby play a role in viral re-activation from sensory neurons. One obvious candidate in this regard is the Brn-3 POU transcription factor originally identified by He et al. [19] and shown to be expressed at high levels in sensory neurons as well as having close identity with the product of the *unc-86* gene which controls sensory neuron development in the nematode [20].

More recent studies, however, have shown that three closely related forms of Brn-3 exist, known as Brn-3a, Brn-3b and Brn-3c [21–23], and each of these is encoded by a distinct gene [23]. These factors are expressed in the ND7 sensory neuron cell line used in our previous studies on the interaction of HSV with neuronal cells [21] as well as in sensory neurons *in vivo* [21,22]. In addition, they have been shown to bind to several octamer/TAATGARAT-related sequences [23] (T. Theil and T. Möroy, unpublished work) and to modulate the activity of artificial test promoters containing such sequences [24,25]. We have therefore tested the effects of these factors on HSV IE-1 and IE-3 promoters in both fibroblasts and neuronal cells.

MATERIALS AND METHODS

Plasmid DNAs

The IE-1 and IE-3 reporter constructs contain the appropriate promoter (from –585 to +150 for IE-1 [26] and from –330 to

+33 for IE-3 [27]) driving expression of the chloramphenicol acetyltransferase (CAT) reporter gene. The Oct-1 and Oct-2.5 expression vectors contain the appropriate full-length cDNAs [28] cloned under the control of the cytomegalovirus IE promoter in the vector pJ7 [29]. The Brn-3a, -3b and -3c expression vectors contain full-length cDNA or genomic clones for each of these proteins [23] cloned under the control of the Moloney murine leukaemia virus promoter in the vector pLTR poly which has been modified by deletion of a cryptic splice site in the simian virus 40 3' untranslated region [24,25].

DNA transfection

Transfection of DNA was carried out by the method of Gorman [30]. Routinely 1×10^6 BHK-21 cells [31] or ND7 cells [32] were transfected with 5 μ g of the reporter plasmid and 5 μ g of the expression vector. In all cases cells were harvested 72 h later. The amount of DNA taken up by the cells in each case was measured by slot-blotting the extract and hybridization with a probe derived from the ampicillin-resistance gene in the plasmid vector [33]. This value was then used to normalize the values obtained in the CAT assay as a control for differences in uptake of plasmid DNA in each sample.

CAT assay

Assays of CAT activity were carried out by the method of Gorman [30] using samples that had been equalized for protein content as determined by the method of Bradford [34].

RESULTS AND DISCUSSION

In initial experiments, we transfected BHK cells lacking endogenous Brn-3 [31] with 5 μ g of expression vectors containing full-length clones of Brn-3a, Brn-3b or Brn-3c [23] and 5 μ g of the IE3-CAT construct which contains the HSV IE-3 promoter (from -330 to +33) driving expression of the CAT gene [27]. All the Brn-3 expression vectors had previously been shown to direct a similar level of protein production in the transfected cells [23,24]. In these experiments (Figure 1a), the IE-3 promoter was clearly transactivated by the expression vectors expressing either

Figure 1 Co-transfection of BHK cells (a) or ND7 cells (b) with the IE-3-CAT plasmid and either the parental expression vector [23] containing the Moloney murine leukaemia virus promoter (MMLV) or the same vector containing full-length clones for Brn-3a, -3b or -3c

Values are expressed relative to the level of promoter activity when IE-3-CAT was co-transfected with the expression vector alone (100%) and are means of two determinations, the range of which is shown by the error bars.

Table 1 Effects of Brn-3 isoforms on the IE-1 promoter

Values are the activity of CAT observed in the indicated cells transfected with IE-1-CAT and either the parental expression vector alone or the same vector containing full-length clones for Brn-3a, -3b or -3c. All values are the mean of two determinations and are expressed relative to the level of promoter activity when IE-1-CAT was transfected with expression vector alone.

Transfected DNA	CAT activity (%)	
	BHK cells	ND7 cells
IE-1-CAT plus		
Vector	100	100
Brn-3a	204	300
Brn-3b	80	88
Brn-3c	117	217

Brn-3a or Brn-3c compared with the level of promoter activity observed with the empty expression vector alone. In contrast, the Brn-3b expression vector reduced the activity of the IE-3 promoter below that observed with the empty expression vector. This result parallels that obtained previously [24,25] in which both Brn-3a and Brn-3c stimulated the activity of artificial promoters containing different added octamer-related motifs whereas Brn-3b repressed these promoters. Most importantly, however, this result indicated that the different forms of Brn-3 can modulate the activity of a natural viral promoter indicating that Brn-3a and Brn-3c may be involved in the stimulation of the IE promoters in neuronal cells.

To investigate this further we determined whether the Brn-3 factors could affect IE-3 promoter activity in neuronal cells that contain endogenous Brn-3 and Oct-2. We therefore transfected the ND7 neuronal cell line which expresses all the different forms of Brn-3 [21] as well as Oct-2 [11,12] with the IE-3-CAT construct and the Brn-3 expression vectors as before. In these experiments (Figure 1b) transactivation of the IE-3 promoter by both Brn-3a and Brn-3c was observed as in the BHK cells, although the effect was marginally less than that observed in BHK cells possibly because of the inhibitory effect of endogenous Oct-2.

It is clear therefore that both Brn-3a and Brn-3c can stimulate the IE-3 promoter when introduced into different cell types. However, whereas the product of the IE-3 gene, ICP4, plays a critical role in lytic infection with HSV [35], the product of the IE-1 gene, ICPO, appears to be critical for re-activation from latency [36]. We therefore transfected BHK and ND7 cells with the Brn-3 expression plasmids and a construct in which the IE-1 promoter (from -585 to +150) drives expression of the CAT gene [26]. In these experiments (Table 1) both the Brn-3a and -3c vectors were able to transactivate the IE-1 promoter, although the effect of Brn-3a was greater than Brn-3c on this promoter, in contrast with the effect on the IE-3 promoter.

These findings indicate therefore that two distinct forms of the Brn-3 transcription factor can activate the HSV IE-1 and IE-3 promoters. This effect is of particular interest as both Brn-3a and Brn-3c are expressed in sensory neurons [22], the natural site of HSV latency *in vivo*, and could therefore potentially play a role in re-activation. Moreover, the fact that these forms can induce the IE promoters in ND7 cells indicates that they can function in the presence of the inhibitory Oct-2 factor.

In order to investigate the relationships between the stimulation of the IE promoters by Brn-3a and -3c and their repression by Oct-2, we utilized BHK cells which do not express endogenous Brn-3 or Oct-2. These cells were transfected with 5 μ g of the IE-

Table 2 Effect of Brn-3 isoforms on the IE-3 or IE-1 promoters in the presence or absence of Oct-2.5

Values indicate the level of CAT activity (mean of two determinations) when cells were transfected with 5 µg of either IE-1-CAT or IE-3-CAT and 5 µg of parental expression vector or the Brn-3a, -3b or -3c expression vectors together with 5 µg of a plasmid expressing Oct-2.5 (+Oct-2.5) or the corresponding parental expressing vector (—Oct-2.5). All values are expressed relative to the level of promoter activity when IE-3-CAT or IE-1-CAT was co-transfected with the expression vectors alone.

	CAT activity (%)			
	IE-3		IE-1	
	—Oct-2.5	+Oct-2.5	—Oct-2.5	+Oct-2.5
Vector	100	15	100	14
Brn-3a	350	50	183	23
Brn-3b	69	14	72	17
Brn-3c	490	110	123	62

3-CAT construct together with 5 µg of one or other of the Brn-3 expression plasmids or empty expression vector and 5 µg of an Oct-2.5 expression plasmid or its corresponding empty expression vector. Oct-2.5 is an alternatively spliced form of the Oct-2 factor which is highly expressed in neurons and has a strong inhibitory effect on the IE promoters [12]. Hence this experiment allows an assessment of the ability of the various forms of Brn-3 to affect IE promoter activity in the presence or absence of the inhibitory activity of Oct-2.5.

In this experiment, as expected, Oct-2.5 had a strong inhibitory effect on the IE-3 promoter, which was stimulated by Brn-3a and Brn-3c (Table 2). Interestingly neither Brn-3a nor Brn-3c was able to overcome entirely the inhibitory effect of Oct-2.5 on the IE-3 promoter. Thus the level of promoter activity in the presence of either of these forms was much lower in the presence of Oct-2.5 than in its absence. However, even in the presence of Oct-2.5 some increase in IE-3 promoter activity was observed with Brn-3a or Brn-3c compared with that observed in their absence.

In order to determine whether these effects extended also to the IE-1 promoter, we co-transfected BHK cells with this promoter and a constant amount of the Oct-2.5 expression vector together with one or other of the Brn-3 expression vectors. In these experiments (Table 2) the Brn-3 expression vectors were unable to reverse the inhibitory effect of Oct-2.5 on the IE-1 promoter. This effect is in contrast with the ability of Brn-3a and Brn-3c to stimulate the IE promoters in ND7 cells which contain endogenous Oct-2 and suggests that the relative levels of Oct-2 and Brn-3 may have a critical role in determining IE promoter activity.

It is likely therefore that in specific neuronal cells, the activity of the IE promoters will depend on the balance between the levels of the stimulatory factors Brn-3a and Brn-3c and the inhibitory effect of Oct-2 and possibly of Brn-3b, the distribution of which in sensory neurons has not been reported. This balance may be different in different sensory neurons, resulting in the two fates that have been reported for sensory neurons after HSV infection *in vivo*; i.e. some cells undergo a productive lytic infection whereas a latent infection is established in the majority [7,37]. In this regard, it is of particular interest that Brn-3c is expressed in only a small subset of sensory neurons [22] and it will clearly be of particular interest to investigate whether these Brn-3c-expressing cells are those that become lytically rather than latently infected with HSV.

Table 3 Effect of Brn-3 isoforms on the transactivation of the IE-1 promoter by Vmw65

Values indicate the level of CAT activity (mean of two determinations) when cells were transfected with 5 µg of Vmw65 expression plasmid together with 5 µg of parental expression vector or of Brn-3a, Brn-3b or Brn-3c expression vectors. All values are expressed relative to the promoter activity observed when IE-1-CAT was transfected alone in the absence of Vmw65.

	IE-1-CAT activity (%)
Vmw65 + Vector	600
Vmw65 + Brn-3a	460
Vmw65 + Brn-3b	370
Vmw65 + Brn-3c	444

In addition to any potential effects of Oct-2 or Brn-3, the activity of the IE promoters is also significantly affected by the Oct-1 protein, which forms a complex with the HSV protein Vmw65 which can transactivate the IE promoters [9,10]. We have previously shown [13] that in BHK cells, which contain endogenous Oct-1, transactivation of the IE promoters by co-transfection of Vmw65 can be abolished by including an Oct-2.5 expression vector in the co-transfections. To determine whether the different forms of Brn-3 could also affect transactivation, we co-transfected BHK cells with the IE-1-CAT construct and a Vmw65 expression vector in the presence or absence of the Brn-3 expression vectors. In these experiments, Vmw65 was, as expected, able to transactivate the IE-1 promoter strongly when co-transfected with expression vector alone (Table 3). Clear transactivation was still observed, however, in the presence of Brn-3a, -3b or -3c vector, although this was reduced in extent. This suggests therefore that, even in the presence of Brn-3, binding of the Oct-1-Vmw65 complex to the TAATGARAT sequence still predominates allowing transactivation to occur, although to a decreased extent which is presumably dependent on low-level binding of the Brn-3b inhibitor or of Brn-3a or Brn-3c which are weaker transactivators than the Oct-1-Vmw65 complex.

Hence in neuronal cells that contain both Oct-1 and Oct-2, the decision whether to enter the lytic or latent phase may involve primarily a balance between the stimulatory effect of the Oct-1-Vmw65 complex and the inhibitory effect of Oct-2 [12,13]. In the neuronal cell types that do not contain Oct-1, however [19], a more critical role is likely to be played by the balance between the different forms of Brn-3 and Oct-2.

Moreover, the role of the activating forms of Brn-3 may be of critical importance during re-activation from latent infection where no Vmw65 is present preventing Oct-1 from strongly transactivating the IE promoters. Under these conditions any fall in the level of the inhibitory Oct-2 factor will allow Brn-3a or Brn-3c to activate the IE promoters, resulting in re-activation. In this regard it is of interest that withdrawal of nerve growth factor, which results in the re-activation of latent infections and expression of the IE-1 and IE-3 genes [14], results in a fall in Oct-2 levels in cultured sensory neurons [38]. Similarly, treatment with cyclic AMP, which also induces re-activation and expression of the IE-1 and IE-3 genes [39], results in a rise in Brn-3a levels [25].

Hence alterations in the balance between the POU family factors that stimulate the IE promoters and those that inhibit them may be responsible for the switch from latency to re-activation. In particular, stimuli that cause a fall in Oct-2 levels

would allow the Brn-3a factor, which is present in the great majority of sensory neurons [22], to bind and stimulate promoter activity leading to re-activation. A similar effect could be produced by a rise in the Brn-3a level which would allow it to overcome the inhibitory effect of Oct-2, as occurs when exogenous Brn-3a is transfected into ND7 cells.

We thank Chris Preston (Institute of Virology, Glasgow University, Glasgow, U.K.) for the IE-3-CAT and Vmw65 plasmids, Saul Silverstein (Department of Microbiology, Columbia University, New York) for the IE-1-CAT plasmid and Thomas Wirth (Centre for Molecular Biology, Heidelberg, Germany) for the Oct-2.5 expression vector. This work was supported by Action Research and the Medical Research Council. Y.-Z. L. was supported by the Countess of Lisburne Studentship from UCL Medical School.

REFERENCES

- 1 Roizman, B. and Sears, A. E. (1987) *Annu. Rev. Microbiol.* **41**, 543–571
- 2 Latchman, D. S. (1990) *J. Exp. Path.* **71**, 133–141
- 3 Stevens, J. G., Wagner, E. K., Devi-Rao, G. B., Cook, M. L. and Feldman, L. T. (1987) *Science* **235**, 1056–1059
- 4 Croen, K. D., Ostrove, J. M., Dragovic, L. J., Smialek, J. E. and Straus, S. E. (1987) *N. Engl. J. Med.* **317**, 1427–1432
- 5 Valyi-Nagi, T., Deshmane, S. L., Spivack, J. G. et al. (1991) *J. Gen. Virol.* **72**, 641–649
- 6 Katz, J., Bodin, T. and Coen, D. M. (1990) *J. Virol.* **64**, 4288–4295
- 7 Speck, P. G. and Simmons, A. (1991) *J. Virol.* **65**, 4001–4005
- 8 Margolis, T. P., Sedarati, F., Dobson, A. T., Feldman, L. T. and Stevens, J. G. (1992) *Virology* **189**, 150–160
- 9 O'Hare, P. and Goding, C. R. (1988) *Cell* **52**, 435–445
- 10 Preston, C. M., Frame, M. C. and Campbell, M. E. M. (1988) *Cell* **52**, 425–434
- 11 Lillycrop, K. A., Dent, C. L., Wheatley, S. C. et al. (1991) *Neuron* **7**, 381–390
- 12 Lillycrop, K. A. and Latchman, D. S. (1992) *J. Biol. Chem.* **267**, 24960–24966
- 13 Lillycrop, K. A., Estridge, J. K. and Latchman, D. S. (1993) *Virology* **196**, 888–891
- 14 Spivack, J. G. and Fraser, N. (1988) *J. Virol.* **62**, 1479–1485
- 15 Wilcox, C. L., Smith, R. L., Freed, C. R. and Johnson, E. M. J. (1990) *J. Neurosci.* **10**, 1268–1275
- 16 Spear, P. G. and Roizman, B. (1980) in *DNA Tumour Viruses* (Tooze, J., ed.), 2nd edn., pp. 615–746, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- 17 Tanaka, M., Lai, J.-S. and Herr, W. (1992) *Cell* **68**, 755–767
- 18 Verrijzer, C. P. and van der Vliet, P. C. (1993) *Biochim. Biophys. Acta* **1173**, 1–21
- 19 He, X., Treacy, M. N., Simmons, D. M., Ingraham, H. A., Swanson, L. S. and Rosenfeld, M. G. (1989) *Nature (London)* **340**, 35–42
- 20 Desai, C., Garriga, G., McIntire, S. L. and Horvitz, H. R. (1988) *Nature (London)* **336**, 638–646
- 21 Lillycrop, K. A., Budhram, V. S., Lakin, N. D. et al. (1992) *Nucleic Acids Res.* **20**, 5093–5096
- 22 Ninkina, N. N., Stevens, G. E. M., Wood, J. N. and Richardson, W. D. (1993) *Nucleic Acids Res.* **21**, 3175–3182
- 23 Theil, T., McLean-Hunter, S., Zornig, M. and Moroy, T. (1993) *Nucleic Acids Res.* **21**, 5921–5929
- 24 Morris, P. J., Theil, T., Ring, C. J. A., Lillycrop, K. A., Mörry, T. and Latchman, D. S. (1994) *Mol. Cell. Biol.* **14**, 6907–6914
- 25 Budhram-Mahadeo, V., Theil, T., Morris, P. J., Lillycrop, K. A., Mörry, T. and Latchman, D. S. (1994) *Nucleic Acids Res.* **22**, 3092–3098
- 26 Gelman, I. H. and Silvestein, S. (1987) *J. Virol.* **61**, 3167–3172
- 27 Stow, N. D., Murray, M. D. and Stow, E. G. (1986) *Cancer Cells* **4**, 497–507
- 28 Wirth, T., Priess, A., Annweiler, A., Zwilling, S. and Oeler, B. (1991) *Nucleic Acids Res.* **19**, 43–51
- 29 Morgenstern, J. P. and Land, H. (1990) *Nucleic Acids Res.* **18**, 1068
- 30 Gorman, C. M. (1985) in *DNA Cloning, A Practical Approach* (Glover, D. M., ed.), vol. 2, pp. 143–190, IRL Press, Oxford
- 31 Macpherson, I. and Stoker, M. (1962) *Virology* **16**, 147–151
- 32 Wood, J. N., Bevan, S. J., Coote, P. et al. (1990) *Proc. R. Soc. Ser. B* **241**, 187–194
- 33 Abken, H. and Reifensrath, B. (1992) *Nucleic Acids Res.* **20**, 3527
- 34 Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- 35 Preston, C. M. (1979) *J. Virol.* **29**, 275–285
- 36 Zhu, X., Chen, J., Young, C. S. H. and Silverstein, S. H. (1990) *J. Virol.* **64**, 4489–4498
- 37 Speck, P. G. and Simmons, A. (1992) *J. Gen. Virol.* **73**, 1281–1285
- 38 Wood, J. N., Lillycrop, K. A., Dent, C. L. et al. (1992) *J. Biol. Chem.* **267**, 17787–17791
- 39 Smith, R. L., Pizer, L. I., Johnson, E. M., Jr. and Wilcox, C. L. (1992) *Virology* **188**, 311–318