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Transcription factors interacting with herpes simplex virus α gene promoters in sensory neurons

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ABSTRACT

Interference with VP16-mediated activation of herpes virus immediate-early (or α) genes is thought to be the major cause of establishing viral latency in sensory neurons. This could be brought about by lack of a key activating transcription factor(s) or active repression. In this study we find that sensory neurons express all important components for VP16-mediated α gene induction, such as the POU transcription factor Oct-1, host cell factor (HCF) and GABPα/β. However, Oct-1 and GABPα/β are only present at low levels and the VP16-induced complex (VIC) appears different. We do not find protein expression of the transcription factor Oct-2, implicated by others as an α gene repressor. The POU factor N-Oct3 (Brn 2 or POU3F2) is also present in sensory neurons and binds viral TAATGARAT motifs with higher affinity than Oct-1, indicating that it may be a candidate repressor for competitive binding to TAATGARAT motifs. When transfected into HeLa cells, where Oct-1 and GABPα/β are highly abundant, N-Oct3 represses model promoters with multimerized TAATGARAT motifs, but fails to repress complete α gene promoters. Taken together our findings suggest that modulation of α gene promoters could contribute to viral latency when low concentrations of the activating transcription factors Oct-1 and GABPα/β prevail. Our data, however, refute the notion that competing Oct factors are able to block α gene transcription to achieve viral latency.

INTRODUCTION

Infections by herpes simplex virus (HSV) proceed via either the lytic or the latent pathways, which differ dramatically in viral gene expression (1,2). In permissive cells viral genes are expressed temporally, beginning with activation of the immediate-early (IE or α) genes followed by the early (E or β) and late (L or γ) classes (3). Transcriptional activation of the α genes, especially the transcriptional regulators ICP4 and ICP0, is required for efficient activation of later classes of genes, which are necessary for viral replication (2). α Gene transcription is primarily the result of activation by the viral tegument protein VP16 (also known as αTIF, Vmw65 or ICP25), which is co-recruited to α promoters together with HCF (host cell factor, also known as CFF or C1 factor; 4–9) by the cellular factor Oct-1 (also termed OTF-1 or POU2F1). The Oct-1–VP16–HCF complex (VIC = VP16-induced complex) is formed on TAATGARAT sequences found in all α gene promoters of HSV. VP16-induced activation from the ICP4 TAATGAGAT motif is weaker than from the ICP0 motif ATGCTAATGATAT (10) which extends 5′ (ATGC) to form a complete consensus octamer site (underlined). Optimal activation of the ICP4 motif is the result of a synergism (10) with factors binding to a GA-rich element known as the CGGAAR site, which is found as a tandem repeat in close proximity to the main TAATGARAT motif in the natural ICP4 promoter (11,12). One of these factors, GABPα, is a member of the Ets transcription factor family, whereas the other, GABPβ, contains ankyrin repeats (13).

In contrast, in latently infected sensory neurons of the trigeminal and dorsal root ganglia (DRG) expression of α genes has not been detected (14, see however 15), leading to the hypothesis that there is a transcriptional block here (16–18) which could be responsible for the establishment and maintenance of viral latency due to abortion of the lytic cycle. Several hypotheses have been put forward to account for this block of α gene transcription. One possibility is that after acute infection of the nerve terminals VP16 fails to reach the nucleus, because of the long distance between the nerve terminal and the soma, and is thus unable to transactivate the α genes. It is unlikely, however, that the lack of α gene expression in latently infected neurons is simply due to the absence of VP16, because overexpression of VP16 in neurons of transgenic mice did not prevent the establishment of latency nor did it induce reactivation (19). It is also possible that other transcription factors necessary for VP16-mediated α gene activation might be lacking in sensory neurons, for example Oct-1, HCF or GABPα/β. Alternatively, it has been suggested that the transcriptional block could be due to a neuron-specific repressor (18). Within the framework of the latter concept Oct-2 isoforms (also known as OTF-2 or POU2F2) have been reported to repress VP16-mediated transcription by competing for the TAATGARAT sites in α gene promoters (20–22). The different Oct-2 isoforms are all derived from the same gene and were originally identified in B cells (23,24) and later also in the nervous system (25,26).

In this study we analysed whether sensory neurons express transcription factors required for VP16-mediated α gene activation and tested candidate repressors that bind to TAATGARAT motifs which could account for latency. We found protein expression of the POU domain transcription factors N-Oct3

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(derived from the *Brn2* gene, also termed POU3F2) and low levels of Oct-1, but no detectable amounts of Oct-2 protein. We also detected GABPα/β at low levels and an HCF form which appears different from that in permissive cells. Since N-Oct3 bound avidly to TAATGARAT sequences, we examined whether it could repress VP16-mediated transcription by ectopic expression in human HeLa cells. We found that it repressed transcription from reporter constructs with isolated TAATGARAT sites, whereas the repressive effect was abrogated when tested in the context of the entire ICP4 or ICP0 regulatory regions. Inclusion of the GA-rich motif was largely responsible for this effect, at least in the case of the ICP4 TAATGARAT sequences. Expression of the Oct-2 factor isoforms Oct2.1 and Oct2.5 yielded similar results on transcription, i.e. repression at isolated TAATGARAT motifs but no repression in the context of the natural promoters.

MATERIALS AND METHODS

Reporter and expression plasmids

The reporter plasmids are based on the OVEC-1 vector (27). Oligonucleotides were synthesized with *Sac*I and *Sal*I protruding ends and multimerized four times into OVEC (27).

Oligonucleotides:
Igκ octamer:
5′-CTCGAGACTTAATAATTTGCATACCCTGAAGGCAGGAG-3′;
3′-TCGAGAGCTCTGAATTATTAAACGTATGGGACTTCCGTCCTC-AGCT-5′.
ICP0 TAATGARAT:
5′-CGAGCCGTGCATGCTAATGATATTCTTTGGG-3′;
3′-TCGAGCTCGGCACGTACGATTACTATAAGAAACCCAGCT-5′.
ICP4 TAATGARAT:
5′-CGAGGATCGGGCGGTAATGAGATGCCATGCG 3′;
3′-TCGAGCTCCTAGCCCGCCATTACTCTACGGTACGCAGCT-5′.
$(CGGAAR)_2$:
5′-CGAGATGCGGAACGGAAGCGGAAACCGCCGGG-3′;
3′-TCGAGCTCTACGCCTTGCCTTCGCCTTTGGCGGCCCAGCT-5′.
$[(CGGAAR)_2$ + TAATGARAT]:
5′-CGAGATGCGGAACGGAAGCGGAAACCGCCGGATCGGGCG-GTAATGAGATGCCATGCGGG-3′;
3′-TCGAGCTCTACGCCTTGCCTTCGCCTTTGGCGGCCTAGCCC-GCCATTACTCTACGGTACGCCCAGCT-5′.

The reporter constructs containing the natural ICP0 and ICP4 promoters [designated nat ICP0(TATA$^-$) and nat ICP4(TATA$^-$) respectively] have been described previously (10). The human N-Oct3 expression plasmid (pEV-N-Oct3; 28) and the VP16 expression plasmid (pCGN-VP16; 8) have been described elsewhere.

DNA transfections and S1 nuclease mapping

HeLa cells were cultured in Dulbecco's minimal essential medium (DMEM; Gibco BRL), 2.5% fetal calf serum (FCS), 2.5% newborn calf serum, 100 U/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine. Mouse dko7 fibroblasts were cultured in DMEM, 10% FCS, 100 U/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine (29). HeLa and dko7 cell transfections were performed by co-transfecting 0.5–3 μg CMV-reference, 3 μg pCGN-VP16, 3 μg OVEC reporter plasmid and up to 10 μg Oct factor expression plasmid by calcium phosphate co-precipitation (27). Salmon sperm DNA was added to equalize the amount of transfected DNA per plate. RNA isolation and S1 nuclease mapping were performed as previously described (27). Signals corresponding to specifically initiated β-globin transcripts were quantified using phosphorimager analysis and normalized to the reference signal. Nuclear extracts were prepared according to Schreiber *et al.* (30).

Sensory neuron cultures and tissue extracts

DRG were collected from 100 mouse pups (post-natal days 2–3) and dissociated with 0.5% collagenase (Worthington Biochemical Corp., Freehold, NJ) and 0.1% trypsin (Sigma, St Louis, MO) for 40 min at 37°C. The DRG were centrifuged and gently triturated in DMEM, 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 50 μg/ml streptomycin, 100 ng/ml 2.5S NGF (Gibco BRL), 10^{-5} M cytosine arabinoside, 10^{-5} M fluorodeoxyuridine, 10^{-5} M uridine. The single cell suspension was plated onto tissue culture plates treated with 50 μg/ml poly-L-lysine and 10 μg/ml laminin (Gibco BRL). The primary cultures were incubated for 2–3 days at 37°C and sensory neurons were harvested by removing the loosely adhered neurons by gentle washing. Non-neuronal cells, consisting mainly of any surviving Schwann cells and fibroblast-like cells, remained adherent to the culture dish. This procedure results in a cell population consisting of >95% neurons (31). Nuclear extracts were prepared according to Schreiber *et al.* (30).

Porcine DRG were collected from two freshly slaughtered adult pigs. The tissue was minced and the nuclear extracts were prepared according to Gorsky *et al.* (32). Mouse brain extracts were prepared from the brain of an adult mouse and processed similarly.

Band shift assays

DNA binding reactions were carried out by incubating 1–10 μg nuclear extract with 4 fmol end-labelled DNA and 2 μg poly(dI–dC) in a buffer containing 4% Ficoll, 20 mM HEPES, pH 7.9, 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.25 mg/ml bovine serum albumin at room temperature for 20 min. Where indicated anti-Oct-1, anti-Oct-2 (30) or anti-HCF antisera (kind gifts of Winship Herr, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY) were added to the nuclear extracts prior to incubation with labelled DNA. The anti-Oct-1 and anti-Oct-2A antisera were raised against the recombinant proteins and were shown to be highly specific and non-cross-reactive towards other Oct factors (30). In other experiments purified bacterially expressed VP16 and human Oct-1 POU domain protein (kind gifts of Ben Luisi, MRC Virology Unit, Glasgow, UK) were added to nuclear extracts prior to addition of the other components. Reaction mixtures were electrophoresed in 4% polyacrylamide gels (acrylamide/bisacrylamide 19:1) in 0.25× TBE that had been pre-run for 2 h at 10 V/cm at room temperature. Signal intensities of the respective bands were quantified by phosphorimager analysis.

RESULTS

Oct factor expression in neurons of the sensory ganglia

To determine the relative abundance of Oct proteins in the natural sites of HSV latency we performed band shift assays with nuclear extracts from purified cultures of mouse sensory neurons. Unlike Western blot analysis, this is a very sensitive assay to determine the functional proportion of DNA binding transcription factors. Oct-1 and N-Oct3 (derived from the *Brn2* gene, also termed

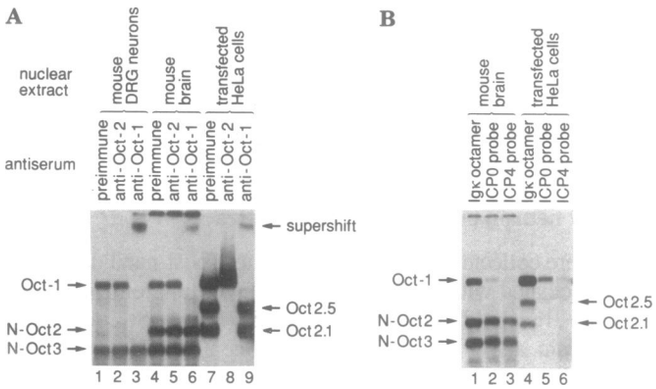

Figure 1. (**A**) Oct factor expression pattern in neural tissues and cross-reactivity with anti-Oct-1 and anti-Oct-2 antisera. Nuclear extracts from purified mouse DRG neurons (lanes 1–3), adult mouse brain (lanes 4–6) and HeLa cells transfected with 10 μg mouse Oct2.1 and Oct2.5 expression plasmids (lanes 7–9) were shifted with ^{32}P-labelled Igκ octamer oligonucleotide (58). Antisera incubations were at a final concentration of 1:100. (**B**) Comparison of Oct factor binding to Igκ octamer, ICP0 and ICP4 TAATGARAT motifs. ^{32}P-Labelled oligonucleotides derived from the Igκ promoter octamer motif, the ICP0 ATGCTAATGATAT and ICP4 TAATGAGAT motifs (see Materials and Methods) were shifted with adult mouse brain extracts (lanes 1–3) or mouse Oct2.1- and Oct2.5-transfected HeLa cell extracts (lanes 4–6).

POU3F2) proteins were detectable in a roughly equimolar ratio using a consensus octamer oligonucleotide probe derived from the Igκ promoter (Fig. 1A). In contrast, Oct-2 isoforms (derived from the *Oct-2* gene) could not be detected in the sensory neuron extracts with the octamer oligonucleotide, which is bound efficiently by two human and mouse Oct-2 isoforms (data not shown and Fig. 1A) nor with the ICP0 or ICP4 TAATGARAT oligonucleotides (Fig. 2A and data not shown), although N-Oct3 (Brn2 or POU3F2) and N-Oct2 (which is derived from the *Brn1* gene and is unrelated to Oct-2, also termed POU3F3) found in mouse brain extracts bound well (Fig. 1B). N-Oct2 and N-Oct3 were originally identified on the basis of their band shift patterns in extracts of mouse and human brain (28,30) and corresponded exactly to the patterns we observed. The identity of the bands was verified using the cloned factors (28). Furthermore, a polyclonal antiserum against human Oct-2A (30) did not cross-react with any of the Oct factors in the sensory neuron extracts, although this antiserum cross-reacted with both mouse Oct-2 isoforms (Fig. 1A). The same Oct factor expression was also seen in nuclear extracts from either adult pig DRG tissue or cultured rat DRG neurons (data not shown). Although sensory neurons have been reported to contain Oct-2, Brn3a (POU4F1) and Brn3c (POU4F3) transcripts (21,33–39), none of the proteins could be detected using these oligonucleotides. As Brn3a and Brn3c are known to bind relatively poorly to these sequences (39,40) they are unlikely to repress HSV α gene transcription from these binding motifs. Thus N-Oct3 and Oct-1 are the major TAATGARAT binding Oct factors expressed in sensory neurons.

Nuclear extracts prepared from adult mouse brain showed a more complex pattern of Oct protein expression. The most abundant were N-Oct2 (Brn1, not related to Oct-2) and N-Oct3, in addition to Oct-1 (Fig. 1A). As with the sensory neuron extracts, we did not observe a strong Oct-2 band in mouse brain extracts using any of our oligonucleotides, although we did detect a faint smear migrating slightly more slowly than mouse Oct2.5 that cross-reacted with the anti-Oct-2 antisera (Fig. 1A). We used these extracts to compare the binding of N-Oct3, N-Oct2 and Oct-1 to different octamer and TAATGARAT-related motifs. The N-Oct factors bound ICP0 and ICP4 TAATGARAT sequences with only slightly reduced affinity compared with the consensus octamer probe (Fig. 1B). As shown before, Oct-1 binding to the TAATGARAT motifs was strongly reduced compared with the consensus octamer probe (10,41–43) and the drop in affinity was even more drastic than in the case of N-Oct2 and N-Oct3 (Fig. 1B). The binding of mouse Oct-2 isoforms (Oct2.1 and Oct2.5) to the TAATGARAT motifs and to the consensus octamer sequence resembled that of Oct-1 (Fig. 1B). Thus Oct-1 and Oct-2 are more selective than mouse N-Oct2 or N-Oct3 in binding to octamer versus TAATGARAT motifs.These results were identical to those obtained with human N-Oct3 (data not shown).

Detection of other positively acting factors in sensory neurons

Band shift analyses with the DRG sensory neuron nuclear extracts were also used to detect other factors involved in VP16-mediated α gene activation. We tested the ability of DRG extracts to support VIC formation with bacterially expressed VP16. Since formation of VIC is strictly dependent upon the presence of both Oct-1 and HCF (4,7,9,44–46, this paper), VIC formation reflects the presence of HCF in the extracts. VP16 formed a complex consisting of three bands with endogenous human Oct-1 from HeLa cells (Fig. 2A, lane 2), but not with mouse Oct-1 from a 3T6 mouse fibroblast cell line (Fig. 2A, lane 5), consistent with previous reports showing that mouse Oct-1 cannot efficiently form complexes with VP16 due to amino acid differences in the POU homeodomain, the site of VP16 interaction (8,47). We therefore used a bacterially expressed human Oct-1 protein consisting only of the POU domain to supplement the mouse extracts. As reported by others, VP16 and the human Oct-1 POU domain protein alone cannot form VIC in the absence of nuclear extract (Fig. 2B, lane 3). Addition of human or mouse extract, however, induced a specific supershifted complex that consisted of three bands (VIC 1, VIC 2 and VIC 3) which contained the bacterially expressed Oct-1 POU domain protein and VP16, as well as HCF supplied by the extract (Fig. 2A, lanes 3, 6 and 15). The presence of HCF was confirmed by addition of an anti-HCF antiserum against human HCF (a kind gift of Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), which abolished complex formation (Fig. 2B). Both the mouse and pig sensory neuron extracts, as well as mouse brain extract, were able to induce VIC formation with VP16 and the human Oct-1 POU domain protein (Fig. 2A, lanes 9, 12 and 15, and Fig. 2C), but not with endogenous mouse or pig Oct-1 (Fig. 2A, lanes 8, 11 and 14). Interestingly, VIC formation in the mouse and pig DRG extracts differed from that of other tissues and cell lines in that the two lower bands (VIC 2 and 3) were predominant, while the upper band (VIC 1) was under-represented or absent (Fig. 2A and C). Moreover, the amount of sensory neuron extract required to support VIC formation was much lower than with 3T6 or HeLa cells, indicating that HCF was relatively abundant in sensory neurons. The fact that porcine DRG showed the same pattern of VIC formation as murine sensory neurons implied that this was a general phenomenon for DRG, rather than a cell culture artefact. These findings show that murine or porcine HCF can substitute

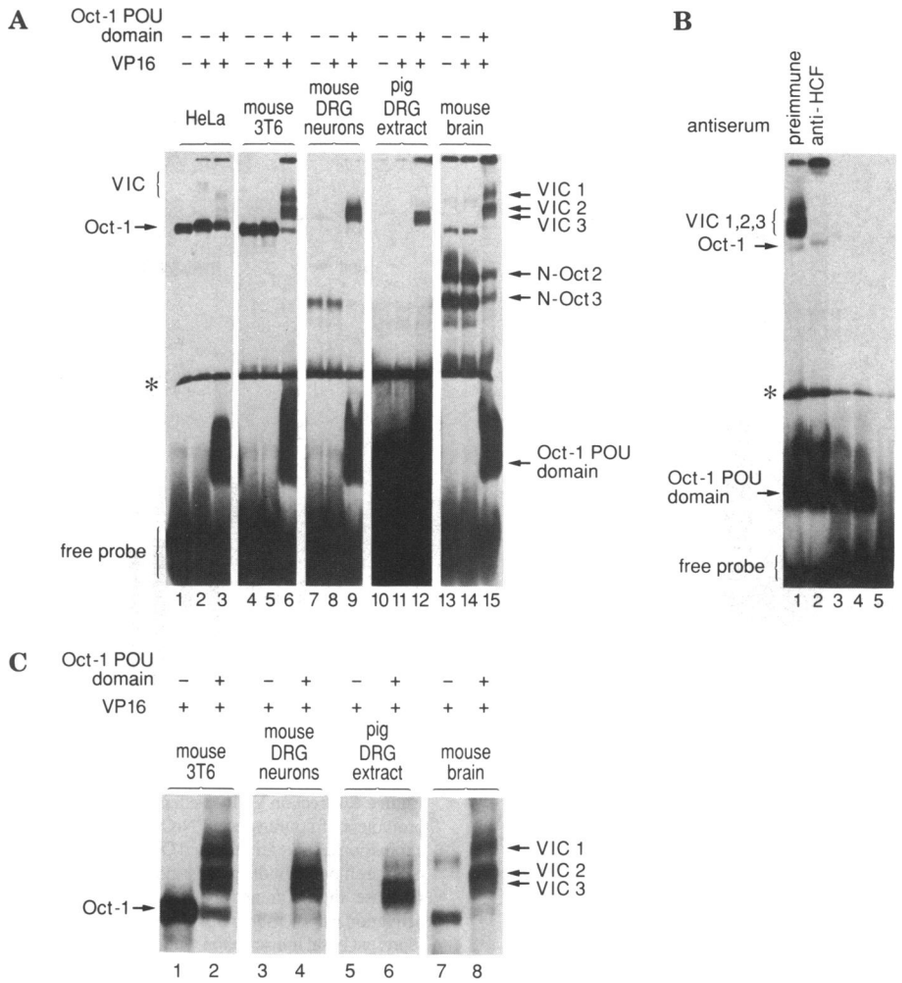

Figure 2. (**A**) Detection of HCF in mammalian cell lines and neural tissues. Nuclear extracts from human HeLa cells, mouse 3T6 cells, cultured mouse DRG neurons, tissue extracts from adult mouse brain and adult pig DRG were shifted with ^{32}P-labelled ICP0 ATGCTAATGATAT oligonucleotide. Extracts were incubated with either 100 ng bacterially expressed VP16 alone or in combination with 10 ng bacterially expressed human Oct-1 POU domain protein. VIC produced with endogenous human Oct-1 from HeLa cells, as well as the VIC1, VIC2 and VIC3 bands produced with the recombinant Oct-1 POU domain protein, are indicated by the arrows. The asterisk indicates a non-specific salt band. (**B**) Inhibition of VIC formation in mouse cells with antisera against human HCF. VIC was formed with 100 ng bacterially expressed VP16, 10 ng human Oct-1 POU domain protein and 3T6 cell nuclear extract and incubated with the ICP0 TAATGARAT oligonucleotide in the presence of pre-immune serum (1:100) (lane 1) or with an anti-HCF antiserum (1:100) (lane 2). VIC is not formed with the Oct-1 POU domain protein and VP16 in the absence of extract (lane 3), with the Oct-1 POU domain protein alone (lane 4) or with VP16 alone (lane 5). (**C**) Expanded view of VIC formation from (A) in mouse 3T6 cells (lanes 1 and 2), mouse sensory neurons (lanes 3 and 4), pig DRG (lanes 5 and 6) and mouse brain (lanes 7 and 8). Recombinant VP16 and Oct-1 POU domain proteins were added as in (A).

for human HCF in VIC formation and that HCF in DRG extracts appears different compared with other tissues or cell lines.

The presence of GABP factors which bind to the CGGAAR motifs present in α gene promoters was also investigated. The use of the $(CGGAAR)_2$ oligonucleotide containing two repeats gave a characteristic double band in HeLa and 3T6 cells (13,48; Fig. 3), which most likely represented formation of single and double GABPα/β heterodimeric complexes. GABPα/β was also highly expressed in brain and gave the same pattern (Fig. 3), however, the cell type of origin could not be determined using these whole tissue extracts. The sensory neuron extracts showed a lower expression level of GABPα/β, with predominant formation of the single GABPα/β complex (Fig. 3). The predominance of the lower band reflected the low level of GABPα/β in these extracts, rather than a modification of these factors, because dilution of HeLa cell extracts also showed preferential loss of the higher band (data not shown).

N-Oct3 does not repress VP16-mediated transcription from the natural ICP0 and ICP4 promoters in human cells

As N-Oct3 was the most abundant TAATGARAT binding protein in DRG sensory neurons we wanted to test whether it could repress VP16-mediated transcription. We used a reconstituted model system consisting of human HeLa cells co-transfected with the human N-Oct3 expression plasmid (28), the reporter constructs containing the entire ICP0 and ICP4 regulatory regions and the VP16 expression plasmid (8). We did not observe repression of VP16-mediated transcription from these promoters

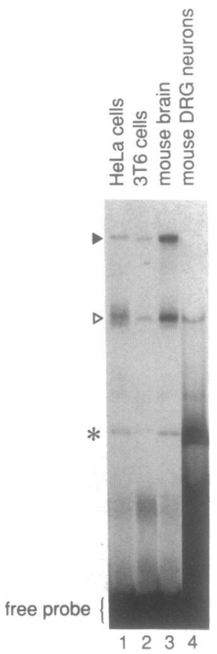

Figure 3. GABPα/β expression in mammalian cell lines and neural tissues. HeLa cell nuclear extract (lane 1), 3T6 cell nuclear extract (lane 2), mouse brain tissue extract (lane 3) and mouse sensory neuron nuclear extract (lane 4) were shifted with ^{32}P-labelled $(CGGAAR)_2$ oligonucleotide. A characteristic double band is seen. The lower band, indicated by the open arrowhead, is a ternary complex consisting of a single GABPα/β heterodimer on a single CGGAAR site. The upper band, indicated by the filled arrowhead, represents double site occupancy by two GABPα/β heterodimers. The asterisk indicates a non-specific salt band.

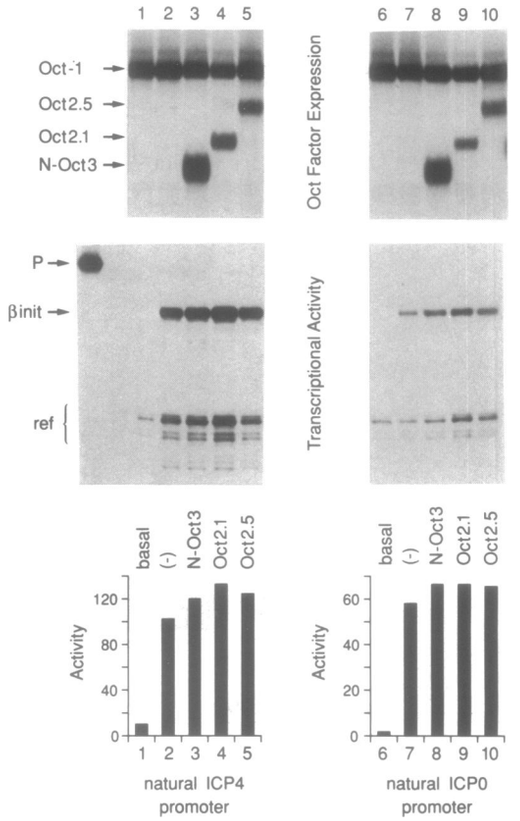

Figure 4. Effect on VP16-mediated transcription from the natural ICP4 or ICP0 promoters of co-transfected N-Oct3, Oct2.1 or Oct2.5. Upper panels show the expression levels of transfected Oct factors using band shift analyses with the Igκ octamer motif. Middle panels show the β-globin transcription products after S1 nuclease digestion and polyacrylamide gel electrophoresis. Transcriptional activation was measured in the presence of VP16, except for lanes 1 and 6, which represent basal transcription without co-transfected VP16. P refers to undigested probe, β-init to correctly inititated β-globin transcripts, and ref indicates the position of the reference signals derived from the co-transfected reference plasmid pCMV-REF. Phosphorimager quantification of correctly initiated transcripts relative to reference signals is shown graphically in the lower panels.

in HeLa cells (Fig. 4), even with high amounts of expressed N-Oct3. Phosphorimager quantification of N-Oct3 relative to Oct-1 showed that the protein levels were comparable with or greater than the amounts present naturally in the sensory neuron or brain extracts (compare Fig. 4 with Fig. 1A). N-Oct3 did not repress or significantly activate basal transcription in the absence of VP16 (2-fold activation with 8 μg pEV-NOct3; data not shown). Since Oct-2 has been proposed by others to repress α gene promoters we also tested mouse Oct2.1 and Oct2.5 in this assay. Neither Oct-2 isoform could repress transcription from the natural ICP0 and ICP4 promoters (Fig. 4). These results were confirmed with Oct-2A, the human homologue of Oct2.1 (data not shown).

N-Oct3 represses VP16-mediated transcription at isolated TAATGARAT motifs

The lack of repression by human N-Oct3 on the natural promoters was surprising because it bound well to TAATGARAT sites, it was highly expressed and it would thus be expected to displace VIC from these sites to impair the VP16 response. Given the complexity of these regulatory regions (49) it seemed possible that neighbouring factors stabilize the VP16 complex. To eliminate these potentially stabilizing effects we tested reporter constructs containing isolated and multimerized TAATGARAT sites from the ICP0 and ICP4 promoters. On these reporters N-Oct3 could indeed repress VP16-mediated transcription (Fig. 5). As previously shown with human N-Oct2 (10), N-Oct3 repressed transcription more effectively from the weak TAATGARAT motif of ICP4 than from the strongly active ICP0 ATGCTAATGATAT motif containing a consensus octamer site. This effect was most probably due to the higher stability of VIC on the ICP0 motif (10). In contrast, the mouse Oct-2 isoforms repressed transcription only slightly from the isolated ICP0 ATGCTAATGATAT motifs and not at all from the ICP4 TAATGAGAT motifs (Fig. 5), reflecting their low affinity for these motifs (Fig. 1B). Thus competing human Oct factors were able to repress VP16-mediated transcription from isolated TAATGARAT motifs in a model system and this repression correlated with their abilities to bind to these sites (Fig. 1B). Taken at face value this argues against a critical role for Oct-2 in HSV α gene repression.

Several laboratories, including ours, have previously shown that the CGGAAR motif confers transcriptional synergy of the VP16-induced response on the ICP4 TAATGARAT motif (10–12). Inclusion of the CGGAAR motifs renders VP16-mediated transcription refractory to N-Oct2 repression, perhaps due to cooperative interactions between GABPα/β and VIC (10). Likewise, increasing concentrations of transfected N-Oct3 plasmid resulted in repression of the [$(CGGAAR)_2$ + TAATGARAT]-OVEC reporter, but the repression only reduced activation by half (Fig. 6), whereas repression from the isolated

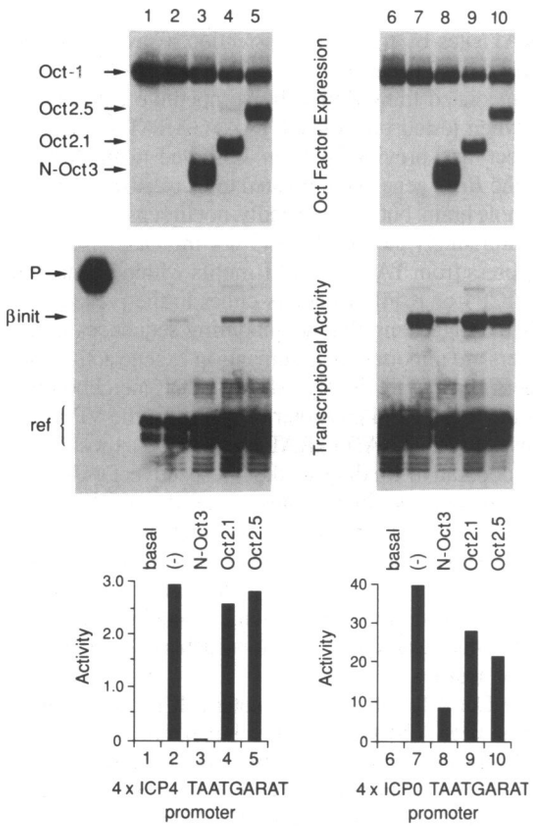

Figure 5. Repression of VP16-mediated transcription from isolated ICP0 ATGCTAATGATAT or ICP4 TAATGAGAT motifs by human NOct-3 or mouse Oct2.1 or 2.5. Upper, middle and lower panels are as described in Figure 4.

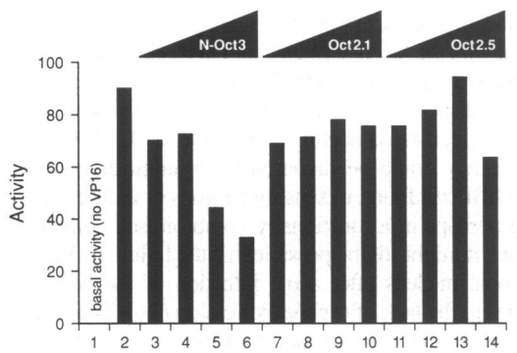

Figure 6. Dose–response characteristics of co-transfected N-Oct3, Oct2.1 and Oct2.5 on VP16-mediated transcription from the [(CGGAAR)$_2$ + TAATGARAT]-OVEC reporter in HeLa cells. Transcriptional activation was measured in the presence of VP16, except for lane 1, which represents basal level transcription. Increasing amounts of N-Oct3 expression plasmid [0.5 μg (lane 3), 1 μg (lane 4), 2 μg (lane 5) or 4 μg (lane 6)] or mouse Oct2.1 or Oct2.5 expression plasmid [2 μg (lanes 7 and 11), 4 μg (lanes 8 and 12), 8 μg (lanes 9 and 13) or 12 μg (lanes 10 and 14)] were co-transfected with VP16 expression plasmid. Phosphoimager quantification is shown graphically as in the lower panel of Figure 4.

ICP4 TAATGARAT motifs resulted in basal level transcription (i.e. without VP16; Fig. 5). The mouse Oct-2 variants were even less effective and did not repress the [(CGGAAR)$_2$ + TAATGARAT]-OVEC reporter construct at all (Fig. 6).

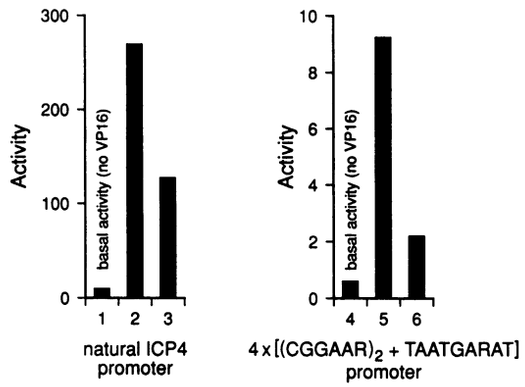

Figure 7. Repression of natural ICP4 promoter (left) and [(CGGAAR)$_2$ + TAATGARAT] (right) reporters by co-transfected N-Oct3 in mouse dko7 cells. Basal level transcription is shown in lanes 1 and 4. Activated transcription with co-transfected VP16 in the absence of N-Oct3 is shown in lanes 2 and 5 and in the presence of 6 μg transfected N-Oct3 in lanes 3 and 6.

N-Oct3 represses VP16-mediated transcription from the natural ICP4 promoter in murine cells

It seemed possible that the repressive effects of Oct factors on the natural α gene promoters observed by others was due to a species difference in the cells used (20–22). Therefore, we tested N-Oct3 for repressive effects in murine dko7 cells (29). N-Oct3 was able to repress VP16-mediated transcription from the natural ICP4 promoter and from the [(CGGAAR)$_2$ + TAAGARAT] promoter more effectively than in HeLa cells (Fig. 7). Addition of a human Oct-1 expression plasmid overcame the repressive effect of N-Oct3 (data not shown). These results are consistent with the fact that murine Oct-1 does not readily associate with VP16 (Fig. 2A), due to amino acid differences in its homeodomain (8,47), and is therefore more sensitive to disruptive influences by competing Oct factors on TAATGARAT sequences.

DISCUSSION

The establishment of latency by HSV in sensory neurons does not require prior viral replication nor α gene expression, suggesting that the pathways leading to productive and latent infections may diverge at a very early stage of the HSV–host interaction (50,51). Some neurons can be productively infected, while in others HSV establishes latency (52,53), which is thought to be the result of a lack of α gene transcription (16–18). This notion is consistent with observations showing that α gene transcripts cannot be detected in latently infected sensory neurons (14) and with the finding that latency is established more readily when mice are infected with the HSV-1 mutant *in*1814 containing transcriptionally defective VP16 (51). A single key viral regulator responsible for the establishment or maintenance of latency has not been described and is unlikely to exist, since virtually any viable HSV deletion mutant (including mutants deleted for the so-called latency-associated transcripts) can establish latency (16). In our studies we have analysed the factors binding to HSV α gene promoter elements in sensory neurons which bear on the possible hypothesis that there may be a lack or limiting amounts of positively acting host transcription factors required for induction of α genes in neurons, thereby forcing latency through an abortive

attempt at lytic infection (1,2). Alternatively, it has been proposed that there may be a neuronal factor which represses α gene activation through binding to TAATGARAT sequences (18,20).

To address the first hypothesis we analysed the protein levels of transcription factors involved in α gene transcription in nuclear extracts of sensory neurons. The factor most often referred to as lacking is Oct-1, because it had been reported, using *in situ* hybridization, that Oct-1 transcripts could not be detected in both neuronal and non-neuronal cells of the sensory ganglia (33,54). The resultant lack of Oct-1 might account for the failure of α gene expression (1,2). The fact that Oct-1 protein is detectable at low levels in DRG tissue explants from pigs might reflect a higher sensitivity of the analyses used in this study. Moreover, the presence of Oct-1 protein in purified cultures of sensory neurons argues for its neuronal origin. We cannot formally rule out the possibility that Oct-1 was generally up-regulated in these cultures due to explantation and culturing conditions (54). However, in sensory neurons cultured in the presence of nerve growth factor (NGF) HSV can still establish latent infections (55), implying that latency can occur in the presence of Oct-1. While Oct-1 may be scarce or absent in another subpopulation of sensory neurons, this might correlate with productive or latent infection by HSV respectively (52,53). However, Roizman and colleagues (quoted in 56) have not observed the overcoming of latency by overexpression of Oct-1 in recombinant viruses.

We also found that sensory neurons express the crucial component HCF, which is required for VIC formation. VIC formed in permissive HeLa or 3T6 cells or brain can be resolved into three distinct bands, whereas in sensory ganglia extracts from mouse and pig the VIC 1 band is barely represented, if at all. This difference may reflect alternative processing of HCF or even the lack of an additional fourth component, which could account for the largest form of VIC. Interestingly, this difference was specific for sensory ganglia and was not observed in the brain. It is tempting to speculate that the observed difference in VIC formation in sensory neurons could represent an inactive complex(es) incapable of α gene activation. Sensory neurons also express GABPα/β, although the levels of these factors are much lower than those found in permissive cells or even brain. Although we have not performed the respective studies in primary human sensory neurons, our findings showing identical results with pig and rodent DRG extracts suggest that these factors and their expression patterns are conserved among different mammalian species. In this context we note that the supershifted complexes (VIC) for pig and mouse DRG showed the same pattern and was tissue specific rather than species specific.

The second hypothesis to account for HSV latency posits that neurons express transcriptional repressors that keep α gene expression low. It has been proposed that an isoform of Oct-2 present in neurons functions as a repressor by binding to TAATGARAT sites (18,20). Our data, however, indicate that N-Oct3 is the major Oct factor in sensory neurons, besides Oct-1, which binds to TAATGARAT motifs, whereas we have been unable to detect Oct-2 protein. Although sensory neurons contain Oct-2 transcripts (21,33), they apparently do not translate the mRNA into protein, due perhaps to incomplete processing of Oct-2 transcripts in the nervous system (A. Tobler, E. Schreiber and A. Fontana, personal communication). On this basis we consider it highly unlikely that Oct-2 could function as a repressor of α gene activation in sensory neurons. Since at least some, if not all, sensory neurons express N-Oct3, we tested the ability of N-Oct3 to function as a repressor of VP16-mediated transcription at TAATGARAT sites by transient transfection assays in human HeLa cells. Our results indicate that N-Oct3 was able to completely repress VP16-mediated transcription by competitive DNA binding, but only when tested on isolated TAATGARAT sequences. The same effect has previously been observed with N-Oct2 (derived from the *Brn1* gene and unrelated to Oct-2; 10), which is expressed in whole brain, but, unexpectedly, not in sensory neurons (this paper). Surprisingly, neither N-Oct3 nor the Oct-2 isoforms were able to repress from TAATGARAT motifs within the natural context of the ICP4 or ICP0 promoters either in the presence or absence of VP16, suggesting that neighbouring sequences in the natural promoters are important determinants in α gene activation. We and others have previously shown that neighbouring CGGAAR motifs in the ICP4 promoter can enhance the VP16-inducible response of ICP4 TAATGARAT sites (10–12), most likely by means of cooperative binding to their respective DNA sites (57). These and perhaps additional sites render the ICP4 TAATGARAT motif less sensitive to repression by competing Oct factors (10, this paper). Thus to achieve complete repression from the natural HSV α gene promoters competing Oct factors would have to overcome these synergistic interactions, which would seem more likely with limiting amounts of factors such as GABPα/β, as found in sensory neurons.

Repression by Oct-2 factors from the natural ICP4 promoter in rodent cells has been observed by Latchman and co-workers (20,22). The divergence of our results from those studies can be explained by a species difference in the cells used. HSV α gene promoters seem to be more readily repressed by Oct factors in rodent cells than in human cells, since rodent Oct-1 interacts only very weakly with VP16 (8,47, this paper). Therefore, it may not form sufficient VIC, even within the context of the natural promoters, to overcome the repressive effect of competing Oct factors. Indeed, when co-transfected into murine cells N-Oct3 could effectively repress the natural ICP4 promoter (Fig. 7). This finding is also consistent with the fact that rodent animal models are less permissive for productive infection by HSV (16).

Our data dispel the notion that latency is established simply by a repressive effect by competing Oct factors at TAATGARAT sites. Rather, latency could be favoured by limiting amounts of Oct-1 and/or GABPα/β found in sensory neurons or a difference in HCF, resulting perhaps in a functionally compromised complex. On the other hand, it might still be possible that the failure of VP16 to reach the neuronal nucleus after acute infection of the nerve terminals contributes to the lack of α gene expression (16). The mere absence of VP16 in the nuclei of infected sensory neurons cannot, by itself, account for the establishment of latency; the data of Roizman and co-workers (19) show that despite the expression of VP16 in sensory neurons, latency is still established and α gene expression is not induced, indicating that there are likely to be multiple controls on latency. It would be interesting to determine if the HCF form predominant in DRG prevents transactivation or whether increased expression of Oct-1 combined with GABPα/β overcomes the neuronal block of α genes.

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