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The herpes simplex virus immediate-early protein ICP27 stimulates the transcription of cellular Alu repeated sequences by increasing the activity of transcription factor TFIIIC

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Infection with herpes simplex virus (HSV) results in an increase in the transcription of the endogenous Alu repeated sequence by RNA polymerase III. This effect is also observed in uninfected cells stably transformed with a plasmid expressing the HSV immediate-early protein ICP27 or in cells transfected with the gene encoding this protein. Both uninfected cells expressing ICP27 and cells infected with virus producing functional ICP27 display increased activity of the cellular transcription factor TFIIIC when compared with untreated cells. This increase is not observed, however, in cells infected with a mutant strain of virus which does not produce ICP27. Hence ICP27 induces elevated Alu transcription by activating transcription factor TFIIIC, which is the limiting factor for such transcription. This is the first report of increased activity of a cellular transcription factor during HSV infection, when most cellular gene activity is inhibited.

INTRODUCTION

Studies on the interaction of viruses with cultured cells can provide important information not only on viral pathogenesis but also on cellular regulatory processes with which viruses interact. In particular, studies on cellular genes induced during viral infection can throw light both on the processes normally regulating these genes as well as on the viral regulatory proteins which induce them. These considerations are of particular importance in the case of herpes simplex virus (HSV), where lytic infection results in a profound repression of most host cell biosynthesis (reviewed in Fenwick, 1984). This process occurs both post-transcriptionally (Sydiskis & Roizman, 1967; Nishioka & Silverstein, 1977, 1978; Inglis, 1982) and by a decrease in the transcription of cellular genes by both RNA polymerase I (Wagner & Roizman, 1969) and RNA polymerase II (Stenberg & Pizer, 1982; Kemp & Latchman, 1988*a*). Interestingly, however, whereas the rate of transcription of 5 S and tRNA genes by RNA polymerase III is unaffected by HSV infection, we have shown that the rate of transcription of the cellular Alu repeated sequence by this polymerase actually increases in HSV-infected cells (Jang & Latchman, 1989).

A study of the interaction of HSV with the Alu repeated sequence is thus of considerable interest, since it may throw light on the interaction of the virus with cellular control systems and possibly on how differences in this interaction in different cell types might result in lytic or latent infection with HSV (reviewed in Roizman & Sears, 1987; Latchman, 1990). This is particularly so in that some of the 100000 copies of the Alu sequence in each human genome are transcribed by RNA polymerase II and others by RNA polymerase III (for review see Rogers, 1985). Thus many of these 300 bp sequences are found within intervening sequences or in the 3′ untranslated regions of protein-coding genes, and are hence transcribed by RNA polymerase II as part of the large primary transcripts of such genes. In contrast, other copies of these repeats contain a functional internal promoter recognized by RNA polymerase III and are transcribed in isolation by this polymerase into small RNA species (Jelinek & Schmid, 1982). In most cell types similar levels of Alu transcription by RNA polymerase II and III are observed.

In our previous study (Jang & Latchman, 1989) we showed that whereas the transcription of Alu sequences by RNA polymerase II declined in HSV infection, the transcription of these sequences by RNA polymerase III increased. The Alu system thus offers an unique opportunity to study the effect of HSV on the transcription of the same sequence by two different polymerases.

Here we show that the increased transcription of Alu sequences in HSV infection is dependent upon the viral immediate-early protein ICP27 and that this protein acts by producing increased activity of the RNA polymerase III transcription factor TFIIIC, which binds to the B box of the internal promoter present in the Alu sequence.

MATERIALS AND METHODS

Cells and viruses

HeLa cells, Vero cells and V27 cells [a cell line derived from Vero cells but stably transformed with a plasmid expressing ICP27 (Rice *et al.*, 1989)] were all grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum. Infections with wild-type HSV-1 strain F (Ejercito *et al.*, 1968), HSV-1 tsK (Preston, 1979) and HSV-1 17×2D (Maclean & Brown, 1987) were carried out using 10 plaque-forming units of virus per cell at 37 °C for strains F and 17×2D and at 39 °C (the non-permissive temperature) for tsK. Cells were harvested 6 h after infection for the preparation of whole cell extracts. For the cycloheximide/actinomycin D experiments, cells were infected with virus in the presence of 50 μg of cycloheximide/ml. After 7 h, the cells were washed five times in medium lacking cycloheximide but containing 2.5 μg of actinomycin D/ml. They were then incubated in actinomycin D-containing medium for 2 h prior to harvesting (Hay & Hay, 1980). Mock-infected cells were similarly treated without addition of virus.

Transfection

Transfections of HeLa cells were carried out as described by Gorman (1985) using 10 μg of DNA/2×10^6 cells on a 90 mm plate. DNA was derived from either pAT153 or a plasmid expressing ICP27 under the control of the simian virus 40 (SV40)

Abbreviations used: HSV, herpes simplex virus; SV40, simian virus 40; DTT, dithiothreitol.

promoter (Everett, 1986). At 48 h after transfection, cells were harvested and nuclei prepared as previously described (Patel *et al.*, 1986).

Nuclear run-on assay

Nuclear run-on assays of transcription were carried out as previously described (Patel *et al.*, 1986) and the labelled products were used to probe dot blots on to which the Alu probes *Blur 11* (Deininger *et al.*, 1981) and a ribosomal DNA probe (Kemp *et al.*, 1986) had been spotted. α-Amanitin was added to the reactions at a final concentration of 2.5 μg/ml. This concentration is sufficient to inhibit the activity of RNA polymerase II without affecting RNA polymerases I and III (Lewis & Burgess, 1982).

Oligonucleotides

Complementary pairs of oligonucleotides containing either the A box or the B box sequence of the Alu clone pCD107 (which contains a functional polymerase III promoter; Duncan *et al.*, 1981) were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. The double-stranded A box oligonucleotide had the sequence (non-transcribed strand) 5′ GCTGGGAGTGGTGGCTCA 3′, while the double-stranded B box oligonucleotide had the sequence (non-transcribed strand) 5′ CAAGAGTTCAAGACCAAC 3′. The mutant B box oligonucleotide had the sequence (non-treated strand) 5′ CAAGACTGAACGATCAAC 3′. Following annealing, the oligonucleotides were labelled by phosphorylation with [γ-^{32}P]ATP and T4 polynucleotide kinase.

DNA mobility shift assay

Whole cell extracts were prepared from mock-infected or infected cells exactly as described by Manley *et al.* (1980). For the DNA mobility shift assay, 10 fmol of labelled oligonucleotide probe was mixed with 3 μl of extract in the presence of 20 mM-Hepes, 5 mM-$MgCl_2$, 50 mM-KCl, 0.5 mM-dithiothreitol (DTT), 4% Ficoll and 2 μg of poly(dI,dC) per 20 μl reaction volume. The reaction was incubated for 40 min on ice before separation of DNA/protein complexes by electrophoresis on a 4% polyacrylamide gel in 0.25 × TBE (1 × TBE = 100 mM-Tris/HCl, 100 mM-boric acid and 2 mM-EDTA, pH 8.3). Gels were run for 2–3 h at 150 V and 4 °C, following pre-electrophoresis for about 2 h before use. Complexes were visualized by autoradiography of the dried gel. In experiments on the effect of phosphatase treatment on TFIIIC binding, extracts were treated with the indicated amounts of acid phosphatase for 30 min at 37 °C. Sodium phosphate (pH 7.9) was then added to 3 mM to inhibit the phosphatase before addition of the probe (Hoeffler *et al.*, 1988).

Fractionation of extracts

Cytoplasmic extracts were prepared from equivalent numbers (1×10^9) of mock-infected cells and virus-infected cells harvested 5 h after infection. Fractionation was carried out as described by Hoeffler & Roeder (1985). A 50 mg sample of each extract at a concentration of 5 mg/ml was applied to separate phosphocellulose columns (Whatman p-11) equilibrated with buffer A (20 mM-Hepes, pH 7.9, 20% glycerol, 0.2 mM-EDTA and 0.5 mM-DTT) containing 0.1 M-KCl (13 mg of protein/ml of bed volume).

Each column was washed with buffer A and the bound protein was successively step-eluted (2 column vol.) with buffer A containing 0.3, 0.6 and 1.0 M-KCl. Fractions equivalent to 20% of the bed volume were collected and the amount of each fraction containing 1 μg of protein was used in gel mobility shift assays.

Polymerase chain reaction

PCR analysis was carried out according to the method of Kawasaki (1990) using 1 μg of total RNA as a template for production of DNA with subsequent amplification (30 cycles) of Alu RNA with an appropriate pair of the A and B box oligonucleotides described above, which should yield a PCR product of 70 bp.

South-western blotting

South-western blotting was carried out by a modification of the method of Schneider *et al.* (1990). Protein samples from mock-infected or infected cells were run on an SDS/polyacrylamide gel and transferred to Hybond C (Amersham). Proteins bound to the filter were denatured with 6 M-guanidine hydrochloride in buffer B (10 mM-Hepes, pH 7.9, 100 mM-KCl, 0.1% Nonidet P40, 1 mM-DTT) for 2 × 15 min periods and then neutralized with five washes with buffer B. Subsequently the filters were blocked in 10 mM-Hepes (pH 7.9)/1 mM-DTT/5% milk powder for 1 h and then washed twice with 10 mM-Hepes/1 mM-DTT. The binding reaction with at least 10^7 c.p.m. of concatamerized, nick-translated site B oligonucleotide was carried out for 2 h in 10 mM-Hepes, pH 7.9, 50 mM-NaCl, 0.1 mM-EDTA, 0.1 mM-DTT and 0.25% milk powder, and was followed by two to three washes in 10 mM-Tris/HCl (pH 7.5)/50 mM-NaCl. All procedures were carried out at room temperature.

RESULTS

In our previous work (Jang & Latchman, 1989) we showed that increased transcription of Alu by RNA polymerase III could be achieved by infection at the non-permissive temperature with the HSV-1 mutant tsK, which synthesizes only the five viral immediate-early proteins (Preston, 1979). In contrast, no induction of Alu transcription was observed in infections with a deletion mutant of HSV-1 which lacks the gene encoding one of these five proteins, ICP27 (MacLean & Brown, 1987). These results strongly suggest that ICP27 is involved in the induction of Alu transcription, but do not eliminate the possibility that another immediate-early protein may also be involved in the effect in conjunction with ICP27, or indeed that another unrecognized mutation in the mutant virus may be responsible for its inability to cause increased Alu transcription.

In order to directly test the role of ICP27 in this effect we co-transfected the Alu clone pCD107, which contains a functional RNA polymerase III promoter (Duncan *et al.*, 1981), with either pAT153 plasmid vector or a plasmid expressing ICP27 (Everett, 1986). The constructs were transfected into BHK-21 cells (Macpherson & Stoker, 1962) which do not express any endogenous Alu repeated sequences (Deininger *et al.*, 1981), allowing the assay to be carried out with no background from endogenous Alu transcription. RNA was isolated from the transfected cells and used to prepare cDNA. When levels of Alu-specific RNA derived from the transfected gene were analysed by using the PCR with Alu-specific primers, a greatly increased level of Alu-specific RNA was detectable in the sample co-transfected with ICP27 compared with that co-transfected with plasmid vector, although both samples showed equal levels of actin RNA (results not shown). Treatment of the RNA samples with RNAase but not with DNAase prior to cDNA synthesis abolished the signal, confirming that it was derived from Alu RNA synthesized in the transfected cells and not from contaminating Alu DNA.

Hence ICP27 in the absence of any other viral protein can up-regulate a co-transfected Alu promoter. In order to test whether ICP27 can also activate endogenous Alu sequences within their normal chromatin structure, we transfected the ICP27-expressing plasmid into HeLa cells and compared the rate of Alu transcription with that in control cells transfected with pAT153 vector. To do this, nuclei were isolated from the transfected cells and transcription was measured using a nuclear run-on assay

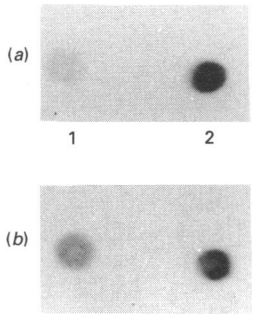

Fig. 1. Nuclear run-on assay of transcription in HeLa cells transfected with either the pAT153 vector (*a*) or a plasmid clone expressing ICP27 under the control of the SV40 promoter (*b*)

Key to spots: 1, Alu clone Blur 11; 2, ribosomal DNA clone.

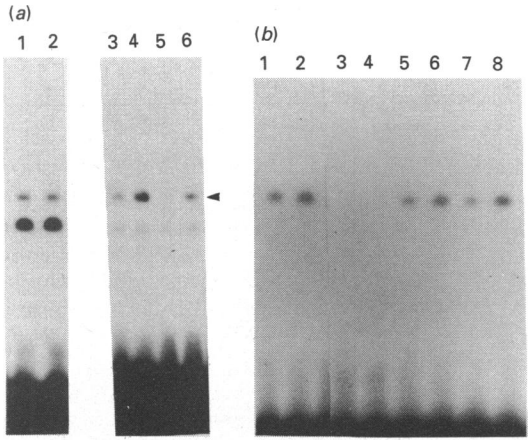

Fig. 2. DNA mobility shift assays

(*a*) DNA mobility shift assay using oligonucleotides derived from either the A box (tracks 1 and 2) or the B box (tracks 3–6) of the Alu promoter. Tracks 1, 3 and 5 show the result with extract prepared from mock-infected HeLa cells; tracks 2, 4 and 6 show results with extracts from infected HeLa cells. In tracks 5 and 6 1 μl of extract was used in the mobility shift assay, whereas the standard 3 μl was used in the other tracks. The arrowhead indicates the sequence-specific complex formed on the B box which increases in HSV infection. The higher-mobility complex formed on this probe is due to a non-sequence-specific DNA-binding protein we have previously described (Kemp *et al.*, 1990; Dent *et al.*, 1991). (*b*) DNA mobility shift assay using labelled B box oligonucleotide in the absence of competitor (tracks 1 and 2) or in the presence of a 400-fold excess of competitor box B oligonucleotide (tracks 3 and 4), of a mutant box B oligonucleotide (tracks 5 and 6) or of an oligonucleotide containing the unrelated octamer motif (tracks 7 and 8). Tracks 1, 3, 5 and 7 show the results using mock-infected cell extracts; tracks 2, 4, 6 and 8 show results with infected cell extracts.

(Patel *et al.*, 1986) which was carried out in the presence of 2.5 μg of the fungal toxin α-amanitin/ml. This level of α-amanitin has been previously shown to be sufficient to inhibit transcription of Alu and other genes by RNA polymerase II, but not by RNA polymerase III (Lewis & Burgess, 1982), and we confirmed this in our previous study (Jang & Latchman, 1989). In these experiments (Fig. 1), clear up-regulation of Alu transcription was observed in the ICP27-transfected cells compared with control cells. Both samples showed the same level of transcription of the control ribosomal clone, which is transcribed by RNA polymerase I and is hence not affected by α-amanitin treatment. As expected, no transcription of the histone H2B gene by RNA polymerase II was observed in these experiments, confirming that this polymerase had been successfully inhibited by the α-amanitin treatment and that we were therefore measuring Alu transcription by RNA polymerase III alone (results not shown).

Hence ICP27 in the absence of any other viral protein can induce increased transcription of endogenous Alu sequences which is sufficiently strong to be observed in these transient transfection experiments where only a minority of cells take up the transfected DNA. A similar ICP27-dependent increase in Alu transcription by RNA polymerase III was also observed in a Vero-derived cell line stably transformed with a plasmid expressing the ICP27 gene (Rice *et al.*, 1989) compared with untransfected Vero cells (results not shown).

To investigate the mechanism by which ICP27 mediates this effect, we prepared whole cell extracts from HeLa cells which had been either mock-infected or infected with wild-type HSV-1. We then investigated the binding of proteins present in these extracts to the RNA polymerase III promoter in the Alu sequence. This promoter contains two elements, the A and B boxes, which together constitute the promoter (Paloella *et al.*, 1983; Perez-Stable *et al.*, 1984: Perez-Stable & Shen, 1986). Accordingly, we synthesized individual complementary pairs of oligonucleotides corresponding to either the A or B box sequence present in the Alu clone pCD107, which contains a functional polymerase III promoter (Duncan *et al.*, 1981). After annealing, the double-stranded oligonucleotides were used in DNA mobility shift assays (Fried & Crothers, 1981) with the infected or mock-infected extracts. In these experiments (Fig. 2*a*, tracks 1 and 2) no change in the binding of proteins to the A box was noted. When the B box was used as a probe, two complexes were observed (Fig. 2*a*, tracks 3–6). The higher-mobility complex was unaffected by HSV infection and is due to a non-sequence-specific DNA-binding protein which we have previously observed forming on several different probes (Kemp *et al.*, 1990; Dent *et al.*, 1991). In contrast, however, increased levels of a lower-mobility complex, formed by the binding of a cellular protein to the B box, were observed in the infected cell extract (arrowed in Fig. 2*a*). This increased binding was readily detectable using two different concentrations of the mock-infected and infected extracts (Fig. 2*a*, tracks 3–6). Binding of this protein was specific to the B box sequence, since binding could be competed for by excess B box oligonucleotide but not by a mutant B box containing several base substitutions at positions conserved between different polymerase III promoters (Cilberto *et al.*, 1983*a*) or by any other unrelated oligonucleotide such as the octamer-binding site for the transcription factor Oct-1 (Fig. 2*b*). The mobility and competition characteristics of the band observed in mock-infected cells were identical to those observed at increased intensity in infected cells. The increased intensity of this band therefore reflects an increased binding activity in infected cells of TFIIIC, which is the only cellular protein known to bind specifically to the B box in both Alu (Perez-Stable *et al.*, 1984; Perez-Stable & Shen, 1986) and other RNA polymerase III promoters (Cilberto *et al.*, 1983*a*,*b*; Lassar *et al.*, 1983; Stillman & Feiduscker, 1984; Baker *et al.*, 1986).

To confirm the identification of this factor as TFIIIC, we fractionated equal amounts (50 mg of total protein) of mock-infected and infected extracts on phosphocellulose columns, as described by Hoeffler & Roeder (1985), and carried out DNA mobility shift assays using the various fractions obtained. This procedure results in the separation of TFIIIC from the other RNA polymerase III transcription factors (Segall *et al.*, 1980; Hoeffler & Roeder, 1985). As expected (Fig. 3), a B box binding activity identical in mobility to that which was elevated in infected cells was obtained in the fraction eluted with 0.6 M-KCl, which is known to contain TFIIIC rather than the TFIIIA-containing flow-through or the TFIIIB-containing 0.3 M-KCl eluate (Hoeffler & Roder, 1985). Approx. 7% of the total protein

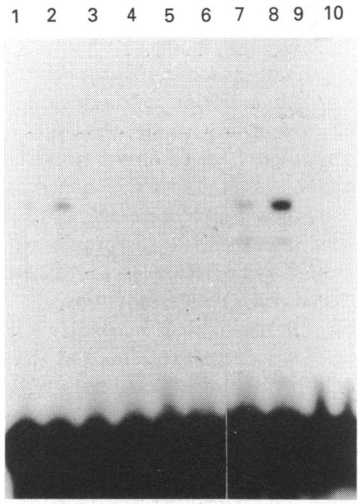

Fig. 3. Partial purification of the B box binding factor, which is increased in infected cells, by phosphocellulose chromatography and subsequent assay by DNA mobility shift with box B oligonucleotide

Tracks 1 and 2 show the result with 5 μg of protein from the total extract prior to fractionation; tracks 3 and 4 show results with the flow-through from the phosphocellulose column, and the other tracks show the results with the 0.3 M-KCl (tracks 5 and 6), 0.6 M-KCl (tracks 7 and 8) and 1 M-KCl (tracks 9 and 10) eluates. A 1 μg sample of protein from each of the fractions was used. Tracks 1, 3, 5, 7 and 9 show the results with the mock-infected extract, and tracks 2, 4, 6, 8 and 10 show results with the infected cell extract.

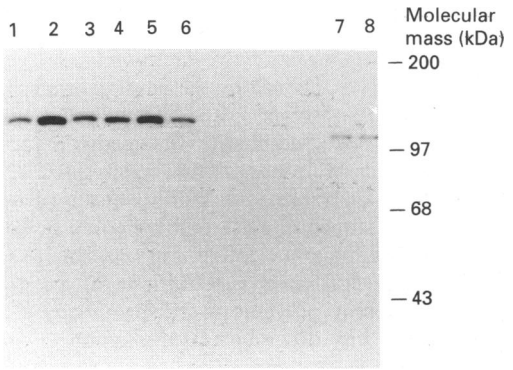

Fig. 4. South-western blotting experiment using a labelled B box oligonucleotide (tracks 1–6) or a labelled octamer oligonucleotide containing a binding site for the 100 kDa Oct-1 transcription factor (tracks 7 and 8)

Extracts were from mock-infected cells (tracks 1, 3 and 7), HSV-1-infected cells (tracks 2 and 8), 17 x 2D-infected cells (track 4) and cells mock-infected (track 5) or HSV-1 infected (track 6) in the presence of cycloheximide and actinomycin D (see the Materials and methods section).

loaded was recovered in the 0.6 M-KCl eluate. Comparison of the amount of binding activity in this fraction (Fig. 3, tracks 7 and 8) with that in the initial extracts (Fig. 3, tracks 1 and 2) indicates that essentially all the binding activity in the initial extract was recovered in the TFIIIC-containing fraction, and that the amount of binding activity observed in this fraction was increased in infected cells. Hence the B box binding activity observed in total cell extracts can be identified as TFIIIC on the basis of its behaviour in fractionation experiments.

To further characterize this factor we carried out South-western blotting to determine the size and abundance of the

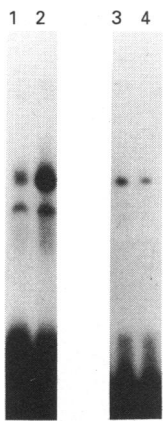

Fig. 5. DNA mobility shift assay using the B box oligonucleotide

Extracts were from cells mock-infected at 39 °C (track 1) or infected with HSV-1 tsK at the non-permissive temperature of 39 °C (track 2), and mock-infected (track 3) or infected with HSV-1 17 x 2D (track 4) at the normal temperature of 37 °C.

protein detected by the B box oligonucleotide in mock-infected and infected extracts. In these experiments (Fig. 4) a labelled B box oligonucleotide detected a 110 kDa protein whose level increased in infected cells (Fig. 4, tracks 1 and 2), whereas a labelled octamer oligonucleotide detected the slightly smaller 100 kDa Oct-1 protein, whose level was unchanged in infected cells (Fig. 4, tracks 7 and 8). The size of the up-regulated protein detected by the B box probe is in agreement with that of the DNA-binding component of TFIIIC previously determined by others (Schneider *et al.*, 1989). Hence the factor which is increased in HSV-infected cells can be identified as TFIIIC on the basis of sequence specificity, size and biochemical characteristics.

To determine whether the change in TFIIIC activity required a full lytic infection with HSV, we carried out infections at the non-permissive temperature with the HSV-1 mutant tsK (Preston, 1979). Due to a defect in the gene encoding the immediate-early protein ICP4, at the non-permissive temperature this virus synthesizes only the abnormal ICP4 protein and the four other immediate-early proteins, and the lytic cycle is aborted at an early stage. As we have previously shown (Jang & Latchman, 1989), this abortive infection is sufficient, however, to induce Alu transcription. In these experiments (Fig. 5), increased binding of TFIIIC to the B box was detected in extracts from tsK-infected cells compared with cells mock-infected at the non-permissive temperature (Fig. 5, tracks 1 and 2) exactly as in infections with wild-type virus. As expected, only the viral immediate-early proteins were observed when cells were labelled with [^{35}S]methionine following infection with tsK at 39 °C confirming that the lytic cycle was not occurring in these cells (results not shown). Hence tsK can induce increased binding of TFIIIC paralleling its ability to induce increased transcription of the Alu sequence.

In contrast, no increase in TFIIIC binding in DNA mobility shift assays (Fig. 5, tracks 3 and 4) or in South-western blotting (Fig. 4, tracks 3 and 4) was observed in cells infected with the HSV-1 mutant 17 x 2D, which does not produce ICP27 (MacLean & Brown, 1987), parallelling the inability of this virus to increase Alu transcription in infection (Jang & Latchman, 1989). This strongly suggests, therefore, that ICP27 is responsible for the increase in TFIIIC binding in infected cells and that this in turn produces the observed increase in Alu transcription.

To confirm the role of ICP27 in the increase in TFIIIC binding, we prepared extracts from V27 cells which had been

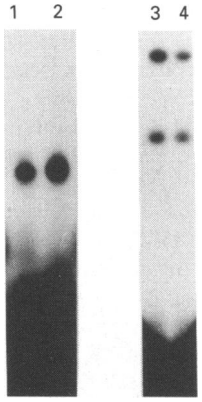

Fig. 6. DNA mobility shift assay using the B box oligonucleotide (tracks 1 and 2) and the unrelated octamer oligonucleotide (tracks 3 and 4)

Extracts were prepared from Vero cells (tracks 1 and 3) or Vero-27 cells (tracks 2 and 4).

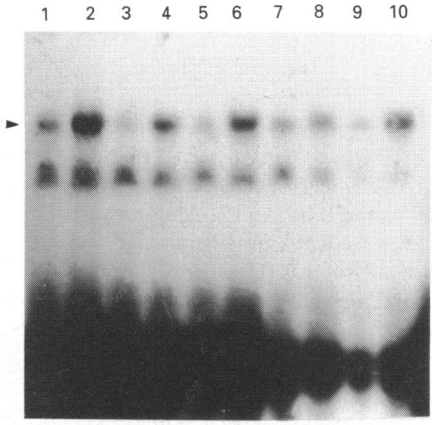

Fig. 7. DNA mobility shift assay using the B box oligonucleotide and mock-infected (tracks 1, 3, 5, 7 and 9) or HSV-1 infected (tracks 2, 4, 6, 8 and 10) HeLa cell extracts

Prior to the DNA mobility shift assay, the indicated amount of acid phosphatase was added to the extracts used in tracks 3 and 4 (0.02 μg), tracks 5 and 6 (0.2 μg), tracks 7 and 8 (2 μg) and tracks 9 and 10 (4 μg). Following incubation at 37 °C for 30 min, sodium phosphate was added to 3 mM to inhibit the phosphatase prior to addition of the labelled oligonucleotide and the subsequent mobility shift assay. Samples in tracks 1 and 2 were similarly treated without addition of phosphatase. The arrowhead indicates the sequence-specific complex found on the B box.

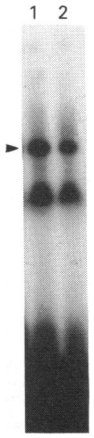

Fig. 8. DNA mobility shift assay using the B box oligonucleotide and cell extracts prepared from mock-infected (track 1) or HSV-1-infected (track 2) HeLa cells

The extracts were prepared from cells either mock-infected or infected under conditions of cycloheximide/actinomycin D reversal (Hay & Hay, 1980). The arrowhead indicates the sequence-specific complex found on the B box.

stably transformed with a plasmid expressing ICP27, and compared the binding activity with that observed in parental Vero cells. As shown in Fig. 6, increased binding of TFIIIC to the B box was indeed observed in V27 cells compared with Vero cells (Fig. 6, tracks 1 and 2). In contrast, no increased binding to the unrelated octamer motif was observed in the V27 cells, confirming that this effect was specific to TFIIIC (Fig. 6, tracks 3 and 4). Hence the increase in Alu transcription in cells transfected with ICP27 (Fig. 1) is parallelled by an increase in TFIIIC activity in such cells.

It is clear, therefore, that the presence in cells of ICP27 alone can increase the binding activity of TFIIIC to the B box in the Alu promoter without the presence of other viral proteins expressed in the infected cell. ICP27 expressed in the infected cell is therefore responsible for this effect in wild-type HSV-1 and tsK infections. In turn, since TFIIIC binding is the limiting factor in polymerase III transcription (Hoeffler *et al.*, 1988; Yoshinaga *et al.*, 1986), such improved binding produces the observed increase in transcription.

It has previously been reported (Hoeffler *et al.*, 1988) that improved TFIIIC binding can be produced by phosphorylation of the protein. To test whether such phosphorylation produced the increased binding observed in infected cells, we treated our extracts with acid phosphatase prior to incubation with labelled B box probe. As shown in Fig. 7, such phosphatase treatment reduced TFIIIC binding in both mock-infected and HSV-1-infected extracts, but the quantitative difference in binding level between the two extracts remained. Similar results were obtained in comparisons of phosphatase-treated mock-infected and tsK-infected extracts or Vero and V-27 extracts (results not shown). Hence ICP27 does not appear to act by changing the phosphorylation state of TFIIIC, but must act either by producing some other modification of TFIIIC which improves binding or by increasing the abundance of this factor.

To investigate this further, we studied whether *de novo* transcription was necessary for ICP27 to induce increased TFIIIC activity. To do this, cells were infected in the presence of cycloheximide, which inhibits protein synthesis. Subsequently cells were washed free of cycloheximide in medium containing actinomycin D, which inhibits all transcription (Hay & Hay, 1980). Under these conditions, the immediate-early mRNAs accumulate during the cycloheximide block but are only translated into their corresponding proteins when the cycloheximide block is removed. The immediate-early proteins made at this stage are capable of modifying cellular components, but cannot induce *de novo* transcription of other viral or cellular genes because of the actinomycin D. In our experiments, on comparing cells infected under these conditions with similarly treated mock-infected cells, no increase in TFIIIC binding in the infected cells was observed in DNA mobility shift assays (Fig. 8) or in South-western blotting experiments (Fig. 4, tracks 5 and 6). As expected, the immediate-early proteins, including ICP27, could be observed by [^{35}S]methionine labelling in cells infected under these conditions at similar levels to those observed early in normal lytic infection. The viral early and late proteins were not synthesized under these conditions, however (results not shown). Hence the increase in TFIIIC binding produced by ICP27 is dependent on the ability of this protein to induce increased transcription,

probably of the gene encoding TFIIIC, thus resulting in increased accumulation of the protein.

DISCUSSION

In this report we have demonstrated that the HSV immediate-early protein ICP27 can increase the binding of transcription factor TFIIIC to the B box of the Alu promoter and up-regulate Alu transcription by RNA polymerase III. Similar effects of the E1A protein of adenovirus (Hoeffler & Roeder, 1985; Yoshinaga *et al.*, 1986) and the immediate-early protein of pseudorabies virus (Gaynor *et al.*, 1985) on TFIIIC binding and polymerase III transcription have previously been observed. In these cases, however, such effects were observed by studying the expression of co-transfected 5S or tRNA genes, and no effect was observed on the endogenous 5S or tRNA genes. This was attributed to the organization of these endogenous genes into stable transcription complexes unable to respond to increased TFIIC activity (Brown, 1984).

In our previous work (Jang & Latchman, 1989) we also observed no increase in endogenous 5S or tRNA gene transcription during HSV infection, in agreement with these studies. Similarly, no increase in the transcription rate of these genes is seen in uninfected cells expressing ICP27 alone (results not shown). We show here, however, that the increased TFIIIC activity induced by ICP27 can produce increased transcription of endogenous Alu repeated sequences (this template was not investigated in the adenovirus or pseudorabies virus studies). This suggests that, unlike endogenous 5S or tRNA genes, endogenous Alu sequences are readily accessible to cellular transcription factors and resemble transfected genes in this regard.

In the case of the increase in TFIIIC binding produced by the E1A protein, different groups have suggested that this is dependent either on a change in TFIIIC phosphorylation producing increased binding (Hoeffler *et al.*, 1988) or on an increase in the abundance of the protein (Yoshinaga *et al.*, 1986). In the case of ICP27, however, the increase in TFIIIC binding cannot be explained by a change in TFIIIC phosphorylation. The ICP27-dependent increase in binding must therefore be produced by some other modification of the protein or by an increase in its abundance. Our finding that the effect of ICP27 is dependent upon its ability to induce *de novo* transcription strongly suggests that ICP27 acts by increasing the abundance of TFIIIC via an increase in the transcription rate of its corresponding gene. This is in agreement with our previous study, which showed that ICP27 can cause increased accumulation of a 40 kDa cellular protein (p40; Estridge *et al.*, 1989) as well as the known ability of ICP27 to induce increased transcription of several viral genes (Sacks *et al.*, 1985; Everett, 1986). Use of mutants lacking various portions of the ICP27 gene would allow the correlation of the ability to induce increased Alu transcription and TFIIIC binding with the effect on p40 levels and on viral gene expression (Rice & Knipe, 1990). It is already clear, however, that the effect on Alu expression and p40 accumulation differs from the effect of ICP27 on viral genes in that the former can be produced by ICP27 alone (see Estridge *et al.*, 1989, for the p40 data). In contrast, the viral late genes which are dependent on ICP27 are not induced in infections (Sacks *et al.*, 1985) or co-transfections (Everett, 1986) unless the immediate-early protein ICP4 is also present.

Such considerations raise the question of the relationship of the effects we observe to the viral life cycle. It is possible that the effect of ICP27 on TFIIIC activity is purely fortuitous, being dependent on a random similarity of the TFIIIC gene promoter to that of a viral gene normally modulated by ICP27. Alternatively, increased TFIIIC activity may be required in HSV-infected cells, possibly for the transcription of specific HSV genes. Although no HSV genes transcribed by RNA polymerase III have been identified, genes encoding small RNAs transcribed by RNA polymerase III do exist both in adenovirus (Hoeffler & Roeder, 1985) and in other herpes viruses such as Epstein–Barr virus and herpes papio (Howe & Shuh, 1988) and similar virally derived small RNAs have been detected in HSV-infected cells (Bachmann *et al.*, 1986). Moreover, one strain of HSV can increase the levels of the cellular La protein (Bachmann *et al.*, 1986; see also Sharpe *et al.*, 1989), which is known to act as an RNA polymerase III transcription termination factor (Gottlieb & Steitz, 1989), indicating that other aspects of the RNA polymerase III system are also enhanced in HSV-infected cells.

Whatever its precise function, the interaction of ICP27 with TFIIIC represents another example of the interaction of HSV regulatory proteins with cellular control systems, which has also been demonstrated for Vmw65 (Kemp & Latchman, 1988*c*; O'Hare & Goding, 1988; Preston *et al.*, 1988) and ICP4 (Latchman *et al.*, 1987; Kemp & Latchman, 1988*b*). Moreover, unlike these other cases, the interaction of ICP27 with TFIIIC results in an increased ability of the transcription factor to bind to its normal cellular sites. This is in contrast to the action of the viral protein Vmw65 on the cellular Oct-1 transcription factor, which allows it to bind to viral TAATGARAT sequences with high affinity (O'Hare & Goding, 1988). These interactions of HSV regulatory proteins and cellular transcription factors and their variation in different cell types may play an important role in the pathogenesis of HSV infection *in vivo*, and in particular on the ability of the virus to produce lytic or latent infection depending on the cell type involved.

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