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The Herpes Simplex Virus Type 1 α Protein ICP27 Can Act as a *trans*-Repressor or a *trans*-Activator in Combination with ICP4 and ICP0

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The herpes simplex virus type 1 (HSV-1) α proteins ICP4, ICP0, and ICP27 are *trans*-acting proteins which affect HSV-1 gene expression. To investigate potential interactions between these α products and to determine the specificity of action of the α proteins in combination with each other compared with their activities individually, we performed a series of transient-expression assays. In these assays we used plasmids containing the α genes encoding ICP4, ICP0, and ICP27 either singly or in combination as effectors and HSV-1 genes of different kinetic classes and heterologous genes as targets. The HSV-1 targets consisted of promoter-regulatory domains from α (ICP0 and ICP27), β (thymidine kinase and alkaline exonuclease), β-γ (glycoprotein D, glycoprotein B, and VP5), and γ (glycoprotein C) genes, each fused to the chloramphenicol acetyltransferase (CAT) gene. The heterologous target genes consisted of the simian virus 40 early promoter with enhancer and the Rous sarcoma virus long terminal repeat promoter and enhancer each fused to the CAT gene. Target promoter activity was measured by the assay of CAT activity in extracts of transfected cells and by Northern (RNA) blot hybridization of CAT mRNA. The results of these experiments showed that ICP4 activated only HSV-1 target genes, whereas ICP0 activated all of the targets and ICP27 had little effect on any of the targets. ICP4 and ICP0 had a synergistic effect when inducing HSV-1 targets, but they did not have this effect on the heterologous targets pSV2-CAT or pRSV-CAT. In fact, lower levels of CAT activity and CAT mRNA were found in the presence of both effectors than with ICP0 alone. Most interestingly, although the effector plasmid containing the ICP27 gene had little effect on its own, two different and marked effects depending on the target were observed when ICP27 was combined with ICP4 or ICP0 or both. A *trans*-repression of the induction seen with ICP4 and ICP0 was found when ICP27 was present in the transfections with pSV2-CAT, pRSV-CAT, pICP0-CAT, pICP27-CAT, pTK-CAT, pgD-CAT, pgB-CAT, and pgC-CAT. This resulted in CAT activity levels which were similar to or lower than the basal level of expression of the target genes in the absence of effector plasmids. This *trans*-repression occurred over a wide range of concentrations of input ICP27 plasmid. In contrast to this repressive effect of ICP27, a *trans*-activation was seen when ICP4, ICP0, and ICP27 plasmids were combined in transfections with pAE-CAT and pVP5-CAT as targets. This *trans*-activation also occurred over a 10-fold range of input ICP27 plasmid. These results suggest that ICP27 can facilitate both down regulation and induction of HSV-1 gene expression.

The regulated expression of mRNA in eucaryotic cells by RNA polymerase II involves promoter selectivity which is mediated in part by cellular transcription factors interacting with *cis*-acting DNA sequences (6, 14, 48, 49, 59, 81). A number of virally encoded *trans*-acting proteins have been shown to be involved in the selective expression of different classes of viral genes in virus-infected cells. The large T antigen of simian virus 40 (SV40), for example, has been shown to be both a negative regulator of SV40 early gene transcription (33, 46, 67, 77, 78, 87) and a positive activator of late gene expression (4, 44, 45). Specific T-antigen-binding sites have been identified in the early promoter region (87), and sites necessary for T-antigen activation have been found in the late promoter (45). In adenovirus, the immediate early protein E1A stimulates early-gene expression. In contrast to the situation found with large T antigen, no unique sites appear to be required for E1A stimulation, only a functional promoter (22, 27, 34, 40, 47, 52, 66). Thus, E1A has been shown to induce heterologous promoters (31, 39, 43) and, furthermore, to negatively regulate enhancer activity (3, 35, 88, 89). Recent studies have shown that E1A may stimulate transcription of early genes by increasing the amount of a cellular transcription factor or by altering or activating this factor (48, 49, 53, 84).

Unlike SV40 or adenovirus, which have only one major *trans*-regulatory protein, herpes simplex virus type 1 (HSV-1) encodes four immediate early or α proteins which have been shown to affect the expression of HSV-1 genes (37, 38). These proteins are termed ICP4 (Vmw 175), ICP0 (Vmw 110), ICP27 (Vmw 63), and ICP22 (Vmw 68). There is an additional α protein, ICP47 (Vmw 12), which does not appear to affect HSV-1 gene expression (54, 57). ICP4 has been shown through the analysis of temperature-sensitive and deletion mutants to be essential for early (β), leaky late (β-γ), and late (γ) gene expression in HSV-1-infected cells (10, 12, 74, 75, 90, 91). ICP4 has also been shown to autoregulate its own expression and that of other α genes (11, 12, 24, 25, 65, 69, 70, 91). ICP0 has been shown to have strong *trans*-inducing activity on HSV-1 immediate early, early, and late promoters in transfection assays (15, 18, 23, 58, 68, 76, 83). ICP27 and ICP22 appear to be involved in late-gene expression, since late-gene products are severely

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diminished in infections with mutants defective in ICP27 or ICP22 (79, 82), although a host cell factor can apparently substitute for the function of ICP22 in some cell lines (82).

The mechanism by which the α products modulate HSV-1 gene expression has not been elucidated. ICP4 is the best characterized α gene product. It binds to sequences in the promoter regions and transcribed noncoding regions of various HSV-1 genes as part of a protein complex (1, 20, 21, 50, 51, 65), and recent evidence suggests that it may bind directly to DNA on its own (64). Partly purified preparations of ICP4 have also been found to stimulate transcription of HSV-1 early and late genes in vitro (1, 73). However, it is not known whether direct binding of ICP4 is necessary for this transcriptional stimulation to occur. In addition, it has not been demonstrated whether the other α proteins interact with specific sequences or whether they increase or modify cellular factors.

It is also possible that α products interact with each other to perform their regulatory functions. It has been demonstrated that ICP4 and ICP0 act synergistically to induce early-gene expression in transfection assays (15, 18, 23, 58, 68, 76), suggesting an interaction between these proteins. Furthermore, Everett (17) has shown that ICP27 enhances the expression of a β-γ gene when ICP4 and ICP0 are also present in the transfections. To investigate interactions between α proteins and to determine the specificity of action of the α proteins in combination with each other compared with their individual activities, we performed a series of transient-expression assays with plasmids containing the α genes for ICP4, ICP0, and ICP27 either singly or in combination with the others. The effects of each of these combinations of effector plasmids were measured by the expression of heterologous genes and HSV-1 genes of different kinetic classes. The results of these experiments show that ICP27 alone had little or no effect on the expression of any of the target genes. However, in combination with ICP4 and/or ICP0, either a marked repression or a stimulation was observed depending on the target gene. Therefore, ICP27 functions as both a *trans*-repressor and a *trans*-activator in the presence of ICP4 and/or ICP0, depending on the target. This suggests that ICP27 interacts with ICP4 and ICP0 and that this interaction can result in transcriptional activation or repression.

MATERIALS AND METHODS

Bacteria and cells. *Escherichia coli* K-12 strain 1100 derivative DH-1 (*recA1 hsdM*$^+$ *nalA96 thi-1 endA supE44* [32]) was used as the host for the propagation of all chimeric plasmids. Rabbit skin fibroblasts (RSF), obtained from the American Type Culture Collection, were grown in Eagle minimal essential medium supplemented with nonessential amino acids, 100 μg of streptomycin per ml, 100 U of penicillin per ml, and 10% fetal calf serum (GIBCO Laboratories).

Plasmids. The effector plasmids containing α genes were constructed as follows. The plasmid pSG28 K/B consists of a 9.0-kilobase (kb) *Bgl*I-*Kpn*I fragment (coordinates 0.79 to 0.86) containing the ICP4 gene cloned into the vector pUC18. It was constructed by first cloning an 8.4-kb *Eco*RI-*Kpn*I fragment into pUC18. The *Eco*RI site occurs 110 base pairs (bp) upstream from the start site of transcription for the ICP4 gene, and so this fragment contained the ICP4 promoter but not the upstream regulatory sequences (8, 55, 56). A 550-bp *Eco*RI-*Bgl*I fragment containing the additional regulatory sequences was ligated to the *Eco*RI-*Kpn*I fragment. pRS1 consists of the 4.8-kb *Sst*I-*Hpa*I fragment (coordinates 0.77 to 0.80) containing the ICP0 gene cloned into pUC18. pSG130 B/S contains the ICP27 gene as a 2.4-kb *Bam*HI-*Sst*I fragment from coordinates 0.745 to 0.762. pBOC2 contains the ICP27 gene with an in-frame stop codon at amino acid 36. This plasmid was constructed by filling in a *Hin*fI site which occurs 105 bp downstream from the ICP27 transcriptional start and inserting a 10-bp *Bgl*II linker. The target plasmids were as follows. The plasmids pSV2-CAT, containing the SV40 early promoter and enhancer, and pRSV-CAT, containing the long terminal repeat from Rous sarcoma virus (RSV) fused to the chloramphenicol acetyltransferase (CAT) gene, were obtained from Bruce Howard (29, 30). The HSV-1 target plasmids were constructed by fusing promoter-regulatory sequences from each HSV-1 gene analyzed to the promoterless construct pSV0-CAT, which was obtained from Bruce Howard (30). Plasmid pICP0-CAT consists of a 900-bp *Sst*I-*Nco*I fragment containing the ICP0 promoter and upstream regulatory sequences as well as the 5′ untranslated leader region (72). This fragment was appropriately modified and inserted into the *Hin*dIII site of pSV0-CAT. pICP27-CAT contains a 325-bp *Bam*HI-*Hin*fI fragment encompassing the ICP27 regulatory region (56) and 55 bp of untranslated leader sequences inserted into pSV0-CAT. Plasmid pTK-CAT consists of the promoter-regulatory sequences of the HSV-1 thymidine kinase (*tk*) gene fused to CAT. It was constructed by inserting a *Kpn*I-*Bgl*II fragment of the *tk* gene from approximately −650 to +50 (61) into the *Hin*dIII site of pSV0-CAT. The plasmid pgB-CAT contains the promoter-regulatory region of the HSV-1 glycoprotein B (gB) gene from an *Xho*I site at −510 to an *Xho*I site at +40 (5, 71) in the *Hin*dIII site of pSV0-CAT. pgD-CAT consists of the promoter region of the glycoprotein D (gD) gene fused to CAT. A fragment from an *Sst*I site at about −400 to a *Hin*dIII site at +1 (92) was inserted into pSV0-CAT. Plasmid pgC-CAT, containing the regulatory sequences of the glycoprotein C (gC) gene, was provided by Fred Homa (36). Plasmids pAE-CAT, containing the alkaline exonuclease gene promoter (AE), and pVP5-CAT, containing the promoter from the major capsid gene VP5, were obtained from E. K. Wagner (9).

Transfection. For transient-expression assays in which CAT activity was assayed, subconfluent monolayers in 35-mm six-well cluster dishes were transfected with 20 μg of target plasmid per ml. Effector plasmids were added at various concentrations in these experiments, and the amounts added are described for each experiment. The total amount of DNA in each transfection was adjusted to 30 μg/ml by the addition of pUC18 DNA, and 1 ml of transfection solution containing the appropriate amount of DNA was added to each well. Transfections were performed as described previously (28), and the cells were shocked with 15% glycerol in minimal essential medium 5 h after the addition of DNA. Cells were harvested 48 h after transfection. CAT activity was assayed as described by Gorman et al. (30). To ensure that measurements were made in the linear range, we used different volumes of extract in the assays for each of the target genes. CAT activity was quantitated by cutting out the spots from the thin-layer chromatograms after autoradiography and then counting them in a liquid scintillation counter.

For measurement of the expression of mRNA, subconfluent monolayers of RSF cells in 75-cm^2 flasks were transfected with 20 μg of target plasmid per ml. Each effector plasmid was added at the concentrations described for each experiment in the figure legends. A total volume of 5 ml of transfection solution was added to each flask, and four

identical flasks were used for each transfection. Cells were shocked with glycerol 5 h after transfection as described above. They were harvested 24 h later.

Northern (RNA) hybridizations. Total RNA was extracted by the guanidium thiocyanate method (7). Poly(A)$^+$ RNA was selected by using oligo(dT)-cellulose (Collaborative Research, Inc.). RNA samples were denatured in glyoxal (63) and fractionated in 1% agarose gels. Transfer to GeneScreen (Du Pont, NEN Research Products) was performed as specified by the suppliers. Blots were prehybridized and hybridized for 18 h in a buffer containing 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 50% formamide as described by Thomas (86). When the hybridizations were performed with a probe specific for CAT mRNA, the blots were incubated at 42°C. When probes specific for ICP0 and ICP27 mRNA were used, hybridizations were performed at 50°C. Dextran sulfate (10%) was included in the prehybridization treatment solution and in the hybridization solution. ^{32}P-labeled probes were nick translated and denatured as described previously (80). The RNA blots were washed once in 2× SSC–0.1% sodium dodecyl sulfate at room temperature and then twice for 30 min each in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C. Hybridized probe was removed before rehybridization with different probes by two successive washes in 0.05× wash buffer (1× wash buffer contains 50 mM Tris [pH 8.0], 2 mM EDTA, 0.5% PP_i, and 0.02% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone [86])–50% formamide for 90 min each at 80°C with constant agitation.

RESULTS

***trans*-Regulation of heterologous and HSV-1 target genes by α gene products.** It has been reported that superinfection with HSV-1 results in induction of heterologous promoters as well as HSV-1 promoters (16, 19, 22). As a first step in analyzing potential interactions between the HSV-1 α gene products ICP4, ICP0, and ICP27 and the specificity of those interactions, we studied the effects of these α gene products on two heterologous promoters, the early promoter with enhancer from SV40 and the promoter and enhancer in the long terminal repeat of Rous sarcoma virus, compared with their effects on an HSV-1 β promoter (*tk*), two HSV-1 β-γ promoters (gD and gB), and an HSV-1 γ promoter (gC). All promoters were fused to the CAT gene (see Materials and Methods). A series of transfection experiments was performed with each of these target genes and with plasmids encoding ICP4, ICP0, and ICP27. The effector plasmids were added in equimolar ratios either singly or in combination. Figure 1 shows the results of these experiments, and the quantitation of the CAT assays is shown in Table 1. In these and all of the following experiments, the results are presented as fold induction over the activity (percent acetylation) seen with the target plasmid in the presence of pUC18 alone with no effector added. This uninduced value was always derived from four separate transfections in each set of assays and was arbitrarily set at 1.0. The fold induction for transfections with effector plasmids was calculated relative to this value. To control for variations in the transfection efficiency, we performed the experiments a number of times, and the means and standard errors are given.

ICP4 induced the HSV-1 target genes, as expected, but had no effect on pSV2-CAT (Fig. 1). This result is in accord with the data of O'Hare and Hayward (70), who showed that ICP4 does not affect expression from the SV40 early promoter and enhancer. ICP0 induced all of the target genes,

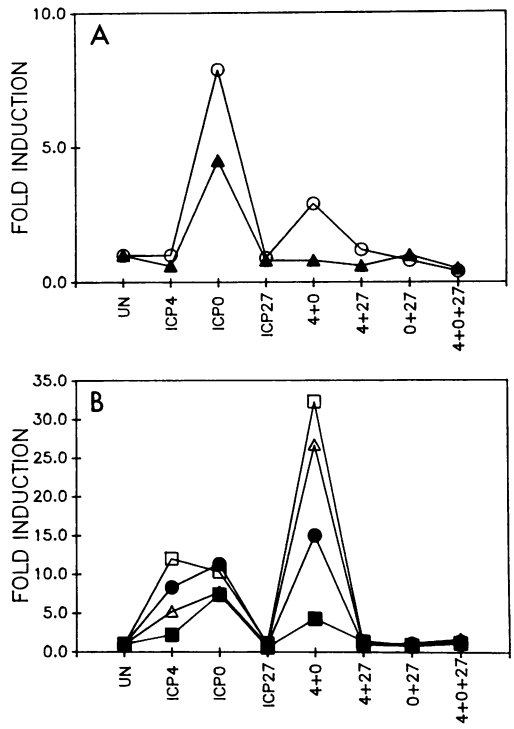

FIG. 1. Effects of equimolar ratios of plasmids encoding ICP4, ICP0, and ICP27 on the expression of heterologous and HSV-1 target genes. Subconfluent monolayers of RSF cells were transfected with heterologous and HSV-1 targets plasmids at a concentration of 20 μg/ml with either pUC18 (UN; 10 μg/ml), pSG28 K/B (ICP4; 4 μg/ml), pRS1 (ICP0; 2.5 μg/ml), or pSG130 B/S (ICP27; 1.5 μg/ml) or with combinations of pSG28 K/B (ICP4), pRS1 (ICP0), and pSG130 B/S (ICP27) as indicated. Cells with harvested 48 h after transfection, and CAT activity was measured (30). The uninduced or basal level of expression for each target was determined in each experiment as the mean level of CAT activity found in four separate transfections with each target plasmid in the presence of 10 μg of pUC18 per ml. The fold induction of CAT activity seen in transfections with effector plasmids was calculated relative to this uninduced level. In this experiment and in all of the following experiments, this uninduced value was set equal to 1.0, and the fold induction relative to the uninduced value is presented. The mean fold induction is presented here. Table 1 shows the number of experiments performed for each target, and the standard errors of the mean are given. (A) Symbols: ○, pSV2-CAT; ▲, pRSV-CAT. (B) Symbols: ●, pTK-CAT; △, pgB-CAT; □, pgD-CAT; ■, pgC-CAT.

and ICP27 had no substantial effect on any of the targets. The most interesting and unexpected finding was that ICP27 in combination with ICP4 or ICP0, or both, significantly repressed the induction found with these effectors when ICP27 was not present. When ICP27 was present, the expression of each of the targets was reduced to the basal level seen in the absence of ICP4 or ICP0 or both. Another unexpected result was that the synergism normally seen with ICP4 and ICP0, which results in greater stimulation of HSV-1 early genes (15, 18, 23, 58, 68, 76), did not occur with pSV2-CAT or pRSV-CAT (Fig. 1A, Table 1). In fact, a 2.5- to 5-fold-lower level of expression was found when ICP4 and ICP0 were present together in the transfections with these heterologous target plasmids compared with transfections in which ICP0 was the only effector. These results suggest that the HSV-1 α products ICP4, ICP0, and ICP27 can interact to

TABLE 1. Effects of equimolar ratios of effector plasmids on heterologous and HSV-1 target genes

Effector plasmid[a]	Fold induction[b] (mean ± SEM [no.]) with:					
	pSV2CAT (no.)	pRSVCAT (no.)	pTKCAT (no.)	pgBCAT (no.)	pgDCAT (no.)	pgCCAT (no.)
Uninduced	1.0	1.0	1.0	1.0	1.0	1.0
ICP4	1.0 ± 0.3 (7)	0.6 ± 0.1 (4)	8.3 ± 2.8 (6)	5.2 ± 0.9 (2)	12.0 ± 7.1 (3)	2.2 ± 0.2 (3)
ICP0	7.9 ± 1.7 (7)	4.5 ± 1.6 (4)	11.3 ± 2.3 (6)	7.7 ± 1.2 (2)	10.3 ± 5.7 (3)	7.4 ± 1.2 (3)
ICP27	0.9 ± 0.2 (7)	0.8 ± 0.1 (4)	0.9 ± 0.2 (6)	0.9 ± 0.7 (2)	1.1 ± 0.4 (3)	0.6 ± 0.1 (3)
ICP4 + ICP0	2.9 ± 1.1 (7)	0.8 ± 0.2 (4)	15.0 ± 3.1 (6)	26.7 ± 5.0 (2)	32.3 ± 13.1 (3)	4.3 ± 0.3 (3)
ICP4 + ICP27	1.2 ± 0.8 (7)	0.6 ± 0.2 (4)	0.9 ± 0.3 (6)	0.9 ± 0.4 (2)	1.0 ± 0.1 (3)	1.4 ± 0.2 (3)
ICP0 + ICP27	0.8 ± 0.3 (7)	1.0 ± 0.3 (4)	1.0 ± 0.1 (6)	1.2 ± 0.8 (2)	0.8 ± 0.1 (3)	0.9 ± 0.3 (3)
ICP4 + ICP0 + ICP27	0.4 ± 0.1 (7)	0.5 ± 0.3 (4)	1.4 ± 0.5 (6)	1.7 ± 0.7 (2)	1.2 ± 0.3 (3)	1.2 ± 0.4 (3)

[a] Effector plasmids used are described in the Materials and Methods and are as follows: ICP4, pSG28 K/B (4 μg/ml); ICP0, pRS1 (2.5 μg/ml); ICP27, pSG130 B/S (1.5 μg/ml). The effector plasmids were added to the transfections at equal molar gene equivalents.

[b] Mean values of induction from the stated number of experiments are presented as fold induction relative to the percent acetylation obtained with the target plasmid in the presence of 10 μg of pUC18 per ml (uninduced level). This level was set at 1.0. The actual uninduced level of acetylation was obtained in each experiment from four separate transfections with each target plasmid. Different amounts of lysates were assayed to ensure that the CAT assays were in the linear range. The average uninduced level of acetylation in these experiments was about 5% for pSV2CAT and pRSVCAT and from 0.4 to 1% for the HSV-1 target plasmids.

negatively regulate gene expression in addition to their previously reported activation functions.

We next wished to ascertain whether the down-regulation seen in the presence of the plasmid pSG130 B/S containing the ICP27 gene was in fact due to the action of the ICP27 protein and not to some other effect such as competition for transcription factors by the ICP27 promoter. Therefore, transfection experiments were performed again with the same HSV-1 target and effector plasmids but with the addition of the plasmid pBOC2, which encodes a truncated ICP27 protein. This plasmid was constructed by inserting an in-frame stop codon at amino acid 36. As in the previous experiment, effector plasmids were added either singly or in combination in equimolar ratios. Addition of plasmid pBOC2 along with the ICP4 and ICP0 plasmids did not result in a significant down-regulation of induction, as is seen with the ICP27 plasmid (Fig. 2). In most instances, the presence of pBOC2 had no effect on the induction of the target plasmid by ICP4 or ICP0 or both, and when a reduction was seen it was on the order of two- to fourfold. In contrast, the presence of the ICP27 plasmid resulted in a 10- to 50-fold reduction in stimulation of expression by ICP4 or ICP0 or both.

The slight level of down-regulation seen with pBOC2 in some of the transfections may have been due to competition for transcription factors by the ICP27 promoter. To test this possibility, we performed a similar set of experiments with a plasmid containing only the promoter-regulatory region of the ICP27 gene. A *Bam*HI (−275)-*Hin*fI (+55) fragment which includes the ICP27 promoter, upstream sequences, and 55 bp of untranslated leader was cloned in pUC18. When this plasmid was added in an equimolar ratio along with the ICP4 or ICP0 plasmid or both, a two- to threefold decrease in the induction of the target gene was seen in some of the transfections (data not shown). Therefore, it is likely that any small decreases seen with pBOC2 were also due to promoter competition for transcription factors or to variability in transfection efficiencies. On the other hand, the substantial decreases seen with the ICP27 plasmid were due to the expression of the ICP27 protein.

In the experiment shown in Fig. 1, it was also noted that the synergistic effect seen with ICP4 and ICP0, which results in enhanced stimulation of HSV-1 targets, was not seen with pSV2-CAT or pRSV-CAT. In fact, a lower level of expression was found when ICP4 and ICP0 were both present than was found with ICP0 as the only effector. To determine whether this effect may have involved competition for transcription factors or whether the ICP4 protein was required, we performed a set of transfections with increasing amounts of the ICP4 plasmid or the same molar gene equivalents of a plasmid containing only the ICP4 promoter (pICP4 promoter). This plasmid contains 660 bp upstream of the ICP4 transcriptional start site, as well as 150 bp of untranslated leader region. Addition of ICP4 along with ICP0 resulted in a 2- to 2.5-fold decrease in the induction seen with ICP0 alone (Table 2). Although this effect is not large, it is reproducible (Table 1, pSV2-CAT), and a similar reduction was not seen with pICP4 promoter. The results with the pTK-CAT target illustrate the effects seen with ICP4 and ICP0 on an HSV-1 early gene, when the *trans*-activating synergism of ICP4 and ICP0 is clearly evident.

The results from these experiments show that the α gene products ICP4, ICP0, and ICP27 can interact to negatively effect the expression of target genes. This *trans*-repression requires the expression of the α proteins and is not due to nonspecific effects such as competition for transcription factors or the presence of some putative plasmid poison sequences.

The α protein ICP27 can act as either a *trans*-repressor or *trans*-activator with different targets. Everett (17) has shown that ICP27 in combination with ICP4 and ICP0 augments the induction of a β-γ target (VP5-CAT) compared with the induction seen with ICP4 and ICP0 alone. Furthermore, in transfections with pTK-CAT and pgD-CAT, he did not observe any repression when ICP27 was added. We have examined a number of parameters to identify the reasons for the differences in our results and those of Everett (17).

The first possibility we investigated was the input concentration of the effector plasmids. This was because the concentrations of transcriptional activators or repressors have been shown to be critical to their action (85). For example, the *lac* repressor acts as a repressor at normal or high concentrations but can also act as an activator at low concentrations by stabilizing RNA polymerase (85). It is possible that the effective concentration of ICP27 expressed in the transfections performed by Everett (17) was significantly lower than in our experiments because Everett used HeLa cells and ICP27 is regulated like a β gene in HeLa cells (25). To investigate this possibility, we used pTK-CAT as a target and added increasing amounts of ICP27 plasmid to the transfections over a 10-fold range. The smallest amount of ICP27 plasmid added was 0.25 μg, which is sixfold less than

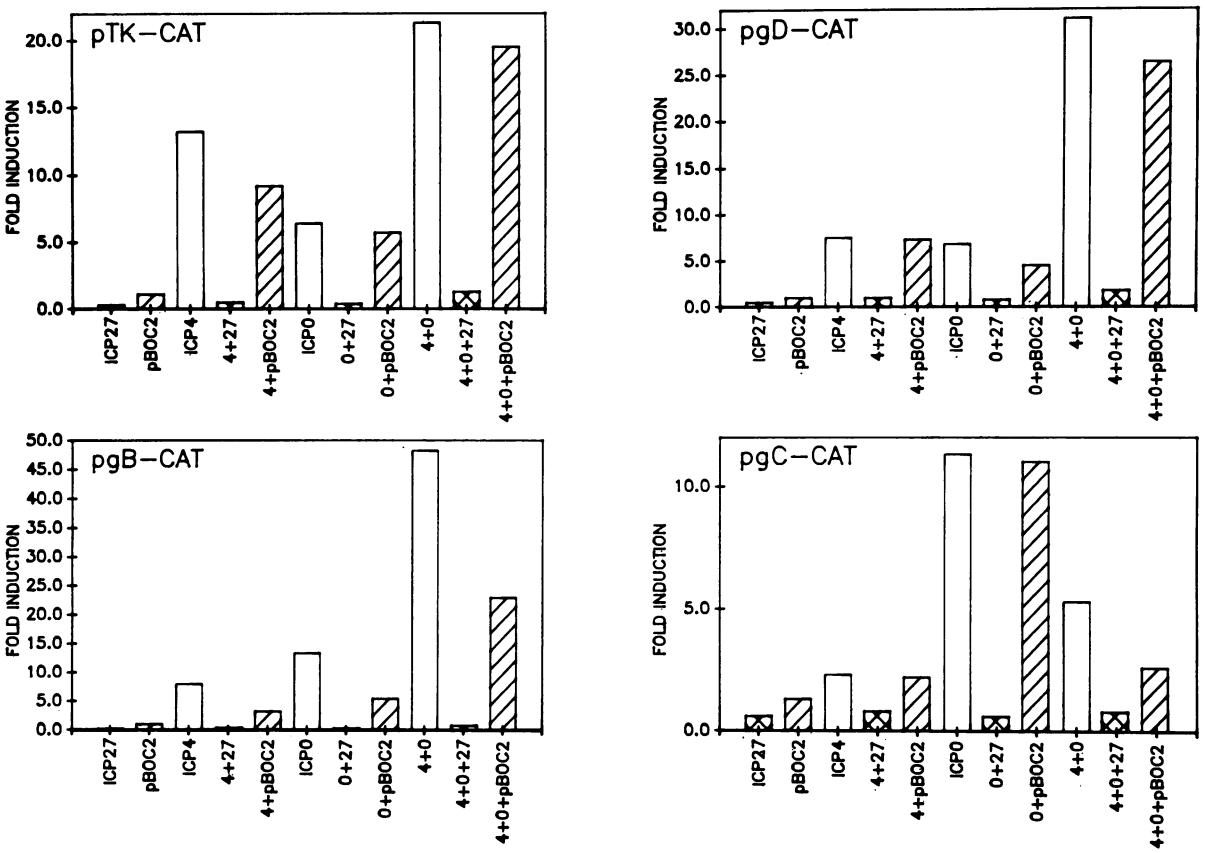

FIG. 2. Requirement of ICP27 protein for down-regulation of induction seen with HSV-1 target plasmids in the presence of ICP4 and ICP0. Four different HSV-1 target plasmids (20 μg/ml) were cotransfected with pSG28 K/B (ICP4; 4 μg/ml), pRS1 (ICP0; 2.5 μg/ml), or pSG130 B/S (ICP27; 1.5 μg/ml). The effector plasmids were added separately or in combination as indicated in each panel. The plasmid pBOC2 was included in these experiments to determine whether the ICP27 protein was required for the down-regulation observed with plasmid pSG130 B/S. pBOC2 contains an in-frame stop codon at amino acid 36 and therefore produces a truncated ICP27 protein. The uninduced level of expression for each target plasmid was again calculated from four separate transfections, and the fold induction in the presence of effector plasmids was calculated relative to this value. The target plasmids are shown in each panel.

the amount added in the experiments performed at equimolar concentrations. The ICP4 and ICP0 plasmids were added at the same concentrations as in the previous experiments. pBOC2 was also included in these experiments, and the concentration was varied over the same range as for pSG130 B/S (ICP27) to be sure that any effects observed were really due to the presence of the ICP27 protein. The addition of ICP27 along with ICP4 or ICP0 or both resulted in a significant decrease in the fold induction found in the absence of ICP27, regardless of the input concentration of the plasmid (Table 3). A similar reduction was not observed with pBOC2, confirming that the ICP27 protein is responsible for the down-regulatory effect. Therefore, even at low concentrations, ICP27 acted as a repressor, with pTK-CAT as a target.

Another possibility for the discrepancy between our results and those of Everett (17) was that the ICP27 gene which we had cloned from HSV-1 strain KOS 1.1 differed in some way from the strain 17-derived clone. To check this, the ICP27 locus from strain 17 was cloned as a *Bam*HI-*Sst*I fragment in pUC18, and an identical set of experiments to those presented in Fig. 2 was performed. Both of the cloned ICP27 genes down-regulated the induction of four different target genes to the same extent (data not shown), indicating that there is no difference between the ICP27 genes cloned from KOS 1.1 and strain 17.

A third possibility for the discrepancy is that ICP27 interacts with some cellular transcription factor which differs between HeLa cells and the RSF cells used in these experiments. This was not tested directly, because we routinely observe much lower transfection efficiencies in HeLa cells. However, a series of transfection experiments was performed with Vero cells and Ltk$^-$ cells with pTK-CAT as the target. The results were identical to those found with pTK-CAT in RSF cells, meaning that ICP27 acted as a *trans*-repressor in all three cell types (data not shown). Although these results do not exclude the possibility of differences in cellular transcription factors which modulate the activity of ICP27, they do indicate that ICP27 acts as a *trans*-repressor in these three different cell lines derived from monkeys, mice, and rabbits.

The fourth possibility we investigated was that ICP27 has different specificities on different target genes. All the targets which we tested up to this point were down-regulated by ICP27, suggesting that there is no specificity in the action of ICP27. This result was surprising, because in vivo analysis of the phenotypes of ICP27 temperature-sensitive mutants has shown that ICP27 is required both for efficient expression of late-gene products and for the turnoff of immediate early gene products (79). Although *trans*-repression of early-gene targets was consistent with the in vivo phenotype observed with ICP27 mutants, *trans*-repression of β-γ (gD-

TABLE 2. Effects of increasing amounts of plasmids containing the ICP4 gene or ICP4 promoter on pSV2CAT and pTKCAT

Effector plasmid[a]	Fold induction[b] with:	
	pSV2CAT	pTKCAT
Uninduced	1.0	1.0
ICP4 (0.25 μg)	1.0	3.0
ICP4 (1.0 μg)	1.1	5.6
ICP4 (5.0 μg)	0.5	7.6
ICP4 promoter (0.25 μg)	1.2	0.6
ICP4 promoter (1.0 μg)	1.6	1.0
ICP4 promoter (5.0 μg)	1.0	0.9
ICP0	5.1	5.6
ICP0 +ICP4 (0.25 μg)	2.6	15.2
ICP0 + ICP4 (1.0 μg)	2.0	22.8
ICP0 + ICP4 (5.0 μg)	1.9	27.6
ICP0 +ICP4 promoter (0.25 μg)	3.9	5.0
ICP0 + ICP4 promoter (1.0 μg)	3.5	3.8
ICP0 + ICP4 promoter (5.0 μg)	4.9	4.6

[a] The plasmid pSG28 K/B containing the ICP4 gene was added to each transfection at the concentrations stated. The plasmid pICP4 promoter containing the ICP4 promoter-regulatory sequences and 150 bp of untranslated leader region was added at equal gene equivalent amounts to those stated for pSG28 K/B. The plasmid pRS1 containing the ICP0 gene was added to the transfections at 2.5 μg/ml.

[b] Values are presented as fold induction over the uninduced level obtained with the target plasmid in the presence of 10 μg of pUC18 per ml. The uninduced level was set at 1.0. The actual percent acetylation obtained for pSV2CAT in this experiment was 4.6%, and for pTKCAT, the percent acetylation was 0.5%.

CAT and gB-CAT) and γ (gC-CAT) target genes was not. Therefore, we performed a series of transfections with target genes which contained promoters representing three kinetic classes. The new targets were pICP0-CAT and pICP27-CAT (α), pAE-CAT (β), and pVP5-CAT (β-γ), and the previously used targets pgB-CAT (β-γ) and pTK-CAT (β) were included for comparison. In these experiments, the input concentrations of the effector plasmids when added singly were varied over a 10- to 25-fold range (Table 4) to observe any differences which could be attributed to concentrations of the effector proteins. When ICP27 was added in combination with the ICP4 or ICP0 plasmids, the input concentrations of the ICP27 plasmid varied from 0.1 to 1.0 μg/ml, whereas the concentration of ICP4 plasmid was always 5 μg/ml and the concentration of the ICP0 plasmid was always 2.5 μg/ml (Table 4).

The results of these experiments (Table 4) confirmed that ICP27 alone had no significant effect on any of the targets but that ICP27 in combination with ICP4 or ICP0 or both had different effects depending on the target gene. The *trans*-repression previously seen with pTK-CAT and pgB-CAT was also observed with the α target genes pICP0-CAT and pICP27-CAT. However, the opposite effect, *trans*-induction, was seen with the β target pAE-CAT and the β-γ target pVP5-CAT. The stimulation was greatest in the presence of all three effector genes. It can also be seen that these targets were not stimulated to levels of expression as high as for the other targets with ICP4 or ICP0, regardless of the input effector plasmid concentration. In fact, no induction of VP5-CAT was observed, except when ICP27 was present in the transfection. These results are similar to those of Everett (17) for the VP5 promoter. This suggests that ICP27 interacts with ICP4 and ICP0 to modulate their activity such that the protein(s) acts as an inducer or repressor depending on the target sequences.

TABLE 3. Effect of different amounts of ICP27 plasmid on pTKCAT expression

Effector plasmid[a]	Mean pTKCAT expression[b] ± SEM
Uninduced	1.0
ICP4	6.3 ± 2.8
ICP4 + ICP27 (0.25 μg)	0.9 ± 0.2
ICP4 + ICP27 (1.0 μg)	0.5 ± 0.2
ICP4 + ICP27 (2.5 μg)	0.4 ± 0.1
ICP4 + pBOC2 (0.25 μg)	7.4 ± 5.1
ICP4 + pBOC2 (1.0 μg)	9.8 ± 5.2
ICP4 + pBOC2 (2.5 μg)	7.6 ± 4.5
ICP0	8.2 ± 1.4
ICP0 + ICP27 (0.25 μg)	1.9 ± 0.7
ICP0 + ICP27 (1.0 μg)	1.0 ± 0.3
ICP0 + ICP27 (2.5 μg)	1.0 ± 0.4
ICP0 + pBOC2 (0.25 μg)	8.1 ± 1.7
ICP0 + pBOC2 (1.0 μg)	9.8 ± 3.9
ICP0 + pBOC2 (2.5 μg)	4.8 ± 1.1
ICP4 + ICP0	12.7 ± 1.8
ICP4 + ICP0 + ICP27 (0.25 μg)	5.3 ± 2.1
ICP4 + ICP0 + ICP27 (1.0 μg)	1.2 ± 1.0
ICP4 + ICP0 + ICP27 (2.5 μg)	0.9 ± 0.5
ICP4 + ICP0 + pBOC2 (0.25 μg)	6.5 ± 0.1
ICP4 + ICP0 + pBOC2 (1.0 μg)	3.5 ± 2.4
ICP4 + ICP0 + pBOC2 (2.5 μg)	5.5 ± 3.6

[a] The plasmid pSG28 K/G containing the ICP4 gene was added at 5 μg/ml to the transfections. The plasmid pSG130 B/S containing the ICP27 gene was added at the concentrations indicated. The plasmid pBOC2, which contains the ICP27 gene and has an in-frame stop codon at amino acid 36, was added at the concentrations indicated. The plasmid pRS1 containing the ICP0 gene was added at 2.5 μg/ml.

[b] Mean values and standard errors of the mean from three experiments are presented as fold induction over the uninduced level obtained with pTKCAT and pUC18 (10 μg/ml). This value (0.7%) was set equal to 1.0.

Measurement of the expression of the α genes in the presence of other α products. The data shown in Table 4 also demonstrated that the α effector plasmids modulated the expression of CAT when driven by the promoters from the ICP0 or ICP27 genes. Both the ICP4 plasmid (pSG28 K/B) and the ICP0 plasmid (pRS1) stimulated the expression of pICP0-CAT and pICP27-CAT. ICP27 alone had no significant (less than twofold) effect on the expression of pICP0CAT or pICP27CAT. However, in combination with ICP4 or ICP0, down-regulation of induction was seen. It has been demonstrated previously that ICP4 can autoregulate its own expression and that of the other α genes (11, 12, 24, 25, 65, 69, 70, 91). Therefore, as levels of ICP4 increase, expression from α promoters decreases. However, we found that this autoregulation requires higher input levels of the ICP4 plasmid than were used in the transfections shown in Table 4. It has also been shown that ICP0 can induce α promoters (24, 70). Because multiple α effector plasmids were cotransfected with the target plasmids in our experiments, some of the regulatory effects which we observed could be the result of a change in the level of an α product in the presence of another α protein. For example, ICP27 could have a negative effect on the expression of ICP4 and ICP0 as well as on the target plasmid. To investigate these effects, we measured the level of expression of ICP0 and ICP27 mRNA in transfection experiments with different target genes and different amounts of input effector plasmids.

In the first experiment, the heterologous target pSV2-CAT was used and the α effector plasmids pSG28 K/B (ICP4), pRS1 (ICP0), and pSG130 B/S (ICP27) were added singly

TABLE 4. Effect of increasing concentrations of effector plasmids on HSV-1 target genes

Effector plasmid[a]	Fold induction with[b]:					
	pICP0CAT	pICP27CAT	pgBCAT	pTKCAT (mean ± SEM)	pAECAT (mean ± SEM)	pVP5CAT (mean ± SEM)
Uninduced	1.0	1.0	1.0	1.0	1.0	1.0
ICP4 (0.25 μg)	1.9	4.3	6.7	3.0 ± 0.2	1.3 ± 0.2	0.7 ± 0.2
ICP4 (0.5 μg)	2.2	2.7	9.7	8.9 ± 1.1	1.0 ± 0.1	1.0 ± 0.5
ICP4 (1.0 μg)	4.4	10.2	8.1	6.4 ± 3.9	1.5 ± 0.6	2.3 ± 1.9
ICP4 (2.5 μg)	2.4	9.0	9.5	7.3 ± 4.7	0.9 ± 0.1	1.9 ± 0.4
ICP4 (5.0 μg)	2.7	18.2	8.8	4.3 ± 1.7	0.8 ± 0.1	1.0 ± 0.2
ICP0 (0.1 μg)	4.4	8.6	6.3	1.9 ± 0.2	3.0 ± 1.4	2.2 ± 0.6
ICP0 (0.25 μg)	3.0	10.4	5.1	3.4 ± 2.0	2.0 ± 0.5	1.8 ± 0.3
ICP0 (0.5 μg)	4.1	7.7	7.7	3.8 ± 1.8	1.2 ± 0.1	1.5 ± 0.3
ICP0 (1.0 μg)	4.4	19.5	9.9	2.7 ± 0.7	3.9 ± 1.9	1.3 ± 0.3
ICP0 (2.5 μg)	3.5	10.0	5.0	4.7 ± 3.1	2.7 ± 0.9	1.9 ± 0.9
ICP27 (0.1 μg)	0.7	2.2	1.1	1.4 ± 1.2	2.0 ± 1.1	1.0 ± 0.1
ICP27 (0.25 μg)	1.4	0.6	1.2	0.5 ± 0.2	0.8 ± 0.3	1.0 ± 0.1
ICP27 (0.5 μg)	1.4	0.7	0.9	0.7 ± 0.5	0.6 ± 0.1	0.9 ± 0.2
ICP27 (0.75 μg)	1.0	0.6	1.1	0.3 ± 0.2	0.9 ± 0.1	1.3 ± 0.3
ICP27 (1.0 μg)	1.2	0.6	1.0	1.0 ± 0.9	0.8 ± 0.1	1.9 ± 0.9
ICP4 + ICP27 (0.1 μg)	4.2	11.1	3.6	2.7 ± 3.0	1.7 ± 0.1	3.9 ± 1.6
ICP4 + ICP27 (0.25 μg)	2.7	3.4	1.7	3.8 ± 2.7	2.9 ± 0.9	5.2 ± 0.9
ICP4 + ICP27 (0.5 μg)	2.4	2.2	2.2	3.3 ± 2.8	2.7 ± 0.5	5.1 ± 1.0
ICP4 + ICP27 (0.75 μg)	1.8	2.1	1.4	1.8 ± 1.5	2.8 ± 0.2	4.3 ± 0.7
ICP4 + ICP27 (1.0 μg)	1.6	1.2	1.4	0.8 ± 0.7	5.6 ± 4.0	6.2 ± 0.4
ICP0 + ICP27 (0.1 μg)	3.4	6.7	2.2	0.8 ± 0.2	2.0 ± 0.4	2.9 ± 2.1
ICP0 + ICP27 (0.25 μg)	1.6	2.2	1.2	1.3 ± 0.8	12.6 ± 7.9	4.5 ± 0.9
ICP0 + ICP27 (0.5 μg)	0.4	1.4	1.1	0.6 ± 0.2	5.2 ± 2.1	4.3 ± 0.3
ICP0 + ICP27 (0.75 μg)	1.4	0.8	0.9	0.8 ± 0.4	3.8 ± 0.7	5.3 ± 1.3
ICP0 + ICP27 (1.0 μg)	1.1	0.9	1.1	1.6 ± 1.2	4.6 ± 2.7	4.6 ± 0.3
ICP4 + ICP0	4.2	12.3	47.5	14.6 ± 7.6	4.3 ± 1.5	6.0 ± 2.8
ICP4 + ICP0 + ICP27 (0.1 μg)	2.8	8.2	22.7	6.5 ± 2.9	7.5 ± 3.6	61.1 ± 32.2
ICP4 + ICP0 + ICP27 (0.25 μg)	5.8	7.4	18.9	4.8 ± 3.2	16.9 ± 7.9	40.1 ± 4.5
ICP4 + ICP0 + ICP27 (0.5 μg)	1.7	4.3	4.6	2.4 ± 1.0	9.2 ± 5.9	44.3 ± 7.3
ICP4 + ICP0 + ICP27 (0.75 μg)	0.9	4.9	2.6	1.9 ± 1.1	9.6 ± 3.1	33.6 ± 2.2
ICP4 + ICP0 + ICP27 (1.0 μg)	1.2	1.9	4.0	0.7 ± 0.4	6.8 ± 1.0	41.8 ± 7.9

[a] The plasmid pSG28 K/B (ICP4) was added at the concentrations indicated, except where pSG130 B/S and pRS1 were also added. In these transfections, pSG28 K/B was added at 5 μg/ml. The plasmid pRS1 (ICP0) was added at the concentrations shown, except where pSG130 B/S and pSG28 K/B were also added. pRS1 was added at a concentration of 2.5 μg/ml to these transfections. The plasmid pSG130 B/S (ICP27) was added at the concentration indicated whether transfected alone or in combination with pSG28 K/B or pRS1.

[b] Values are presented as fold induction over the uninduced level of acetylation obtained with the target plasmids and pUC18 (10 μg/ml). The values shown for pTKCAT, pAECAT, and pVP5CAT are mean values from three experiments, and standard errors of the mean are given.

and in combination in equimolar ratios. Total RNA was extracted 30 h after transfection, and poly(A)$^+$ RNA was selected by oligo(dT) column purification. The Northern (RNA) blot shown in Fig. 3 was hybridized with a probe specific for CAT mRNA and then stripped and rehybridized with a probe specific for ICP0 mRNA. Appropriate exposures of the autoradiograms were scanned with a laser densitometer, and relative peak areas are given. For CAT mRNA, the value obtained with pSV2-CAT alone in the absence of effector plasmids (lane 1) was set equal to 100, and all other values were calculated relative to this value. In the autoradiogram showing ICP0 mRNA, the value obtained with pSV2-CAT and the ICP0 plasmid and no other effectors was set equal to 100, and, again, other values were calculated relative to this value. Figure 3 shows that the level of CAT mRNA expressed in the presence of ICP4 (lane 2), ICP0 (lane 3), or ICP27 (lane 4) alone correlated well with the levels of activity measured in the CAT assays (Fig. 1; Table 1). This was also true when the effector plasmids were added in combination (Fig. 3, lanes 5 to 7). Therefore, the level of CAT activity accurately represents the level of CAT mRNA. In addition, it can be seen that the level of ICP0 mRNA was not substantially altered by the presence of ICP4 (lane 5) or ICP27 (lane 7) in this experiment. Therefore, down-regulation of CAT mRNA levels seen in combinations of ICP0 and ICP4 (lane 5) or ICP0 and ICP27 (lane 7) compared with the level expressed with ICP0 alone (lane 3) was not due to lowered expression of ICP0 mRNA.

We next studied mRNA levels from transfections with HSV-1 target genes (Fig. 4). The targets were pICP0-CAT (α), pTK-CAT (β), and pgC-CAT (γ). ICP0 and ICP27 were the effectors added, and the input concentrations were varied and are described for each experiment. In Fig. 4A, pICP0-CAT was transfected alone (lane 1) or with 0.25 μg (lane 2) or 2.5 μg (lane 3) of pRS1 per ml. First, it can be seen that the level of ICP0 mRNA was much lower than the level of CAT mRNA (the autoradiogram in which the CAT probe was used was exposed for 3 days compared with 18 days for the ICP0 mRNA). A possible explanation for this finding is competition for transcription factors between the ICP0 pro-

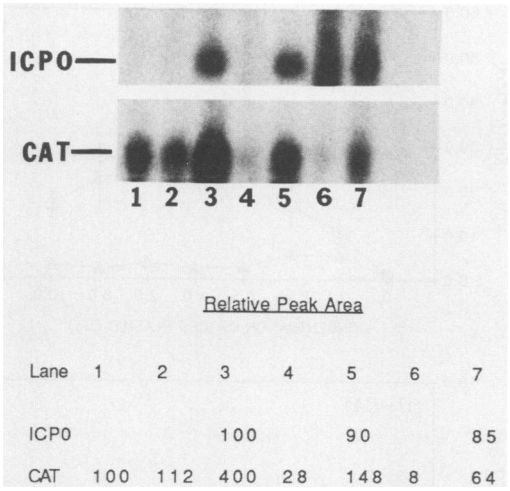

FIG. 3. Expression of ICP0 mRNA in transfections with pSV2-CAT and α effector plasmids. RSF cells were transfected with pSV2-CAT (20 μg/ml) and effector plasmids containing the α genes for ICP4 (pSG28 K/B; 4 μg/ml), ICP0 (pRS1; 2.5 μg/ml), and ICP27 (pSG130 B/S; 1.5 μg/ml) as described in Materials and Methods. Transfection buffer (5 ml) containing the appropriate amounts of DNA was added to each flask. The total DNA concentration was adjusted to 150 μg/75-cm^2 flask by adding pUC18 DNA when necessary. Four identical flasks were set up and used for each transfection. Transfections shown in each lane are as follows: lane 1, uninduced pSV2-CAT; lane 2, pSV2-CAT and pSG28 K/B (ICP4 gene); lane 3, pSV2-CAT and pRS-1 (ICP0 gene); lane 4, pSV2-CAT and pSG130 B/S (ICP27 gene); lane 5, pSV2-CAT with pSG28 K/B and pRS-1; lane 6, pSV2-CAT with pSG28 K/B and pSG130 B/S; lane 7, pSV2-CAT with pRS-1 and pSG130 B/S. Poly(A)$^+$ RNA was denatured in glyoxal (63) and fractionated on a 1% agarose gel. RNA was transferred to GeneScreen. In the upper panel, the blot was hybridized with a 1.3-kb *Nru*I-*Sal*I fragment from within exon 3 of the ICP0 gene (72). The fragment was labeled by nick translation as described previously (28, 80). In the lower panel, the blot was hybridized with a 1.6-kb *Hin*dIII-*Bam*HI fragment encompassing the CAT gene (30). Hybridization with the CAT probe was performed first at 42°C as described in Materials and Methods. After autoradiography, the blot was then stripped and rehybridized at 50°C with the probe specific for ICP0 mRNA. Appropriate exposures of the autoradiographs were scanned with an LKB densitometer. The level of ICP0 mRNA seen in lane 3 in the absence of other α effector plasmids was set at 100%. The remaining values, where the other α genes were also present (lanes 5 and 7), were calculated relative to this level. For CAT mRNA, the level seen in lane 1, where no α plasmids were present, was set at 100%, and all other values were calculated relative to this level.

moter fused to the CAT gene and the ICP0 plasmid. pICP0-CAT was added at the target concentration of 20 μg/ml, whereas the ICP0 plasmid (pRS1) was added at levels that were 8- (lane 3) to 80-fold (lane 2) lower. Second, it can be seen that the amount of stimulation found was not directly proportional to the input effector plasmid concentration. A 1.5-fold increase in CAT mRNA was seen when the ICP0 plasmid (pRS1) was added at 0.25 μg/ml (lane 2), but addition of 10 times more effector plasmid (lane 3) resulted in only a 2.5-fold-higher CAT mRNA level. Addition of the ICP27 plasmid (pSG130 B/S) at 0.25 μg/ml (lane 4), 1.0 μg/ml (lane 5), or 2.5 μg/ml (lane 6) resulted in a lower expression of ICP0 mRNA as well as of CAT mRNA. In fact, ICP0 mRNA could barely be detected on the autoradiograms in the lanes in which ICP27 was present (lanes 4 to 6), and CAT mRNA could not be detected at all. In contrast to the results shown in Fig. 3, in which ICP27 did not significantly lower

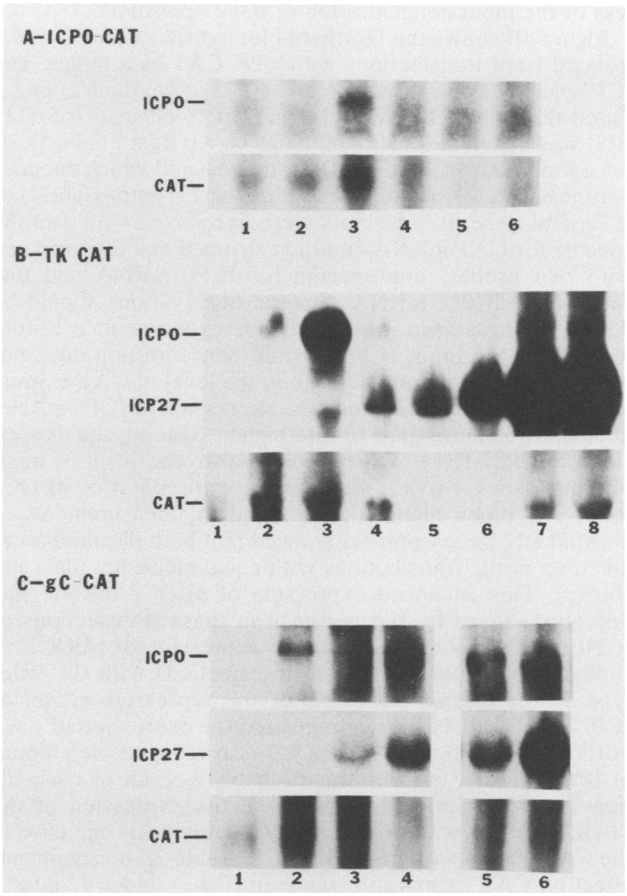

FIG. 4. Northern hybridization analysis of the expression of CAT mRNA and α effector RNA in transfections with three different HSV-1 target genes representing three different kinetic classes. Total RNA was isolated from transfections with HSV-1 target plasmids added at 20 μg/ml and different concentrations of effector plasmids. (A) pICP0-CAT was the target, and hybridization to ICP0 mRNA and CAT mRNA is shown. In lane 1, pICP0-CAT was present along with 10 μg of pUC18 per ml; in lane 2, 0.25 μg of pRS1 (ICP0) per ml was added; and in lane 3, 2.5 μg of pRS1 per ml was added. In lanes 4 to 6, 2.5 μg of pRS1 per ml was added along with 0.25 μg (lane 4), 1.0 μg (lane 5) or 2.5 μg of pSG130 B/S (ICP27) (lane 6) per ml. (B) pTK-CAT was added at 20 μg/ml along with pUC18 at 10 μg/ml (lane 1) or pRS1 at 0.25 μg/ml (lane 2) or 2.5 μg/ml (lanes 3 to 8). pSG130 B/S was added to the transfections at 0.25 μg/ml (lane 4), 1.0 μg/ml (lane 5), or 2.5 μg/ml (lane 6). pBOC2 was added to the transfections shown in lane 7 (0.25 μg/ml) and lane 8 (1.0 μg/ml). (C) Transfections with pgC-CAT are shown. Lane 1 shows the hybridization to RNA from transfections with pgC-CAT and pUC18, whereas pRS1 was added at 2.5 μg/ml to the transfections shown in lanes 2 to 6. The ICP27 plasmid pSG130 B/S was added at 0.25 μg/ml (lane 3) and 1.0 μg/ml (lane 4) to the transfections shown in lanes 3 and 4, whereas pBOC2, the plasmid encoding a truncated ICP27 protein, was present at 0.25 μg/ml (lane 5) or 1.0 μg/ml (lane 6). The blots were first hybridized with a 1.6-kb *Hin*dIII-*Bam*HI probe specific for CAT mRNA. After autoradiography, the blots were stripped and rehybridized with a 1.3-kb *Nru*I-*Sal*I probe from exon 3 of the ICP0 gene (panels A and C) or a mixture of the probe specific for ICP0 mRNA and a 1.2-kb *Bam*HI-*Sal*I probe specific for ICP27 mRNA (panel B). Following autoradiography, the blot in panel C was again stripped and rehybridized with the ICP27 probe.

the ICP0 mRNA levels, in this experiment ICP27 down-regulated both CAT mRNA and ICP0 mRNA levels, regardless of the input concentration of ICP27 plasmid.

Figure 4B shows the Northern blot hybridizations of RNA isolated from transfections with pTK-CAT as a target. The ICP0 plasmid (pRS1) was added at 0.25 μg/ml (lane 2) or 2.5 μg/ml (lanes 3 to 8), whereas the ICP27 plasmid (pSG130 B/S) was added at 0.25 μg/ml (lane 4), 1.0 μg/ml (lane 5), or 2.5 μg/ml (lane 6). Also, pBOC2, the plasmid which encodes a truncated ICP27 protein, was added at 1.0 μg/ml (lane 7) or 2.5 μg/ml (lane 8). The blots were hybridized with a probe specific for CAT mRNA and then stripped and rehybridized with two probes, one specific for ICP0 mRNA and one specific for ICP27 mRNA. Several observations should be noted. First, as seen in the previous experiment, a 10-fold increase in the input ICP0 plasmid concentration does not result in a proportional increase in the level of CAT expression (lanes 2 and 3), although the expression of ICP0 mRNA in lane 3 was more than 10-fold higher. Second, the expression of ICP27 RNA was proportional to the level of input plasmid (lanes 4 to 6), whereas the expression of pBOC2 RNA was much higher (lanes 7 and 8). Measurements of plasmid DNA copy number showed that both plasmids were taken up in the transfections with equal efficiency (data not shown). This enhanced expression of pBOC2 mRNA was probably caused by the presence in these transfections of ICP0, which induced the level of expression of pBOC2. A similar result was not seen in transfections with the wild-type ICP27 plasmid, because of the repressive action of ICP27. Third, ICP27 down-regulated the expression of CAT mRNA and ICP0 mRNA. pBOC2 also reduced the amount of ICP0 mRNA, but this was probably a result of competition for transcription factors, since the expression of the pBOC2 mRNA was so high. As was previously the case in the CAT assays with pBOC2 (Fig. 2; Table 2), no significant effect on CAT expression was seen (Fig. 4, lanes 7 and 8).

In the experiment shown in Fig. 4C, pgC-CAT was the target and the ICP0 plasmid was added at 2.5 μg/ml in lanes 2 to 6, whereas the ICP27 plasmid was added at 0.25 μg/ml (lane 3) and 1.0 μg/ml (lane 4) and pBOC2 was added at 0.25 μg/ml (lane 5) and 1.0 μg/ml (lane 6). The blot was hybridized first with the CAT probe, then stripped and rehybridized with the probe for ICP0 mRNA, and then stripped and rehybridized with the ICP27 probe. As in the previous experiment, the level of pBOC2 mRNA was higher than for the ICP27 mRNA, although both plasmids were added at the same concentrations. This again suggests that ICP0 stimulated the expression of pBOC2. The presence of ICP27 resulted in a decrease of CAT mRNA (lanes 3 and 4), but although ICP0 mRNA levels were decreased, the reduction was less than was seen in the experiment with pTK-CAT.

Three conclusions can be drawn from these experiments. First, the level of CAT mRNA correlated with the level of CAT activity seen previously. Second, the amount of induction found with ICP0 was not proportional to the input ICP0 plasmid concentration or to the level of expression of ICP0 mRNA, suggesting that ICP0 may be acting catalytically to enhance transcription. Third, ICP27 had a negative regulatory effect on ICP0 expression, as well as on the expression of the target plasmid. The extent of this down-regulation varied in different experiments.

The level of induction by ICP0 is not directly proportional to the amount of input plasmid added. To investigate the relationship between the relative amount of ICP0 plasmid added to the transfections and the fold induction of CAT expression, we performed a set of transfection experiments

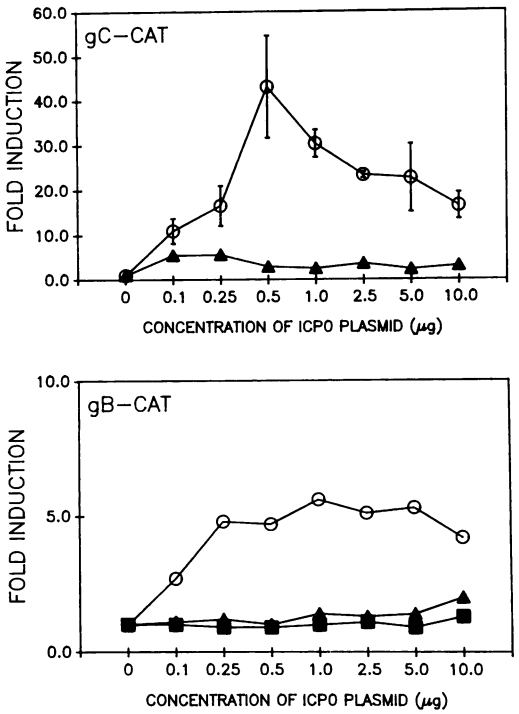

FIG. 5. The level of induction by ICP0 is not proportional to the input ICP0 plasmid concentration. Transfections with pgC-CAT (upper panel) and pgB-CAT (lower panel) were performed in the presence of increasing amounts of the ICP0 plasmid pRS1. In the upper panel, results with pgC-CAT and pRS1 (○) are given as the mean of four separate transfections, and error bars showing the standard errors of the mean are indicated. The lower panel shows the results with pgB-CAT and increasing concentrations of pRS1 (○). The plasmid encoding ICP27 (pSG130 B/S) was added to the transfections with pgC-CAT (upper panel) and increasing amounts of pRS1 at 1.0 μg/ml (▲) and to transfections with pgB-CAT and pRS1 (lower panel) at 0.25 μg/ml (▲) or 1.0 μg/ml (■). Values are presented as fold induction over the induced level of CAT activity found with the target plasmids in the presence of pUC18 and no effector plasmids.

in which the input concentration of pRS1 was varied over a 100-fold range from 0.1 to 10.0 μg/ml. Figure 5 shows the results obtained with pgC-CAT or pgB-CAT as targets. For pgC-CAT, the mean of four separate experiments is plotted, and error bars indicating standard errors of the mean are shown. In both cases, it can be seen that the fold induction of CAT activity was not directly proportional to the amount of input plasmid. Even at very low concentrations (0.1 to 0.25 μg), substantial levels of induction were observed, and these levels were nearly equivalent to levels seen when 20- to 50-fold-larger amounts of ICP0 plasmid were added. These results are similar to those seen in Table 4 and to the Northern hybridization experiments, in which levels of CAT mRNA did not increase proportionally to the increase in ICP0 mRNA expression (Fig. 4A and B). This suggests that the down-regulation of target expression seen when ICP0 and ICP27 are both present is not solely due to a down-regulation of ICP0, because very low levels of ICP0 expression still result in substantial induction of CAT activity. Furthermore, when the ICP27 plasmid was added to the transfections, either at 0.25 μg/ml or at 1.0 μg/ml, a similar level of down-regulation was seen, regardless of the input level of the ICP0 plasmid. Interestingly, a 2.5-fold-higher

level of induction of CAT activity with pgC-CAT was seen in the presence of ICP27 at the lowest concentrations of the ICP0 plasmid (0.1 and 0.25 μg) than was seen when pRS1 was present at 10- to 100-fold-higher concentrations. This result suggests that some minimal amount of ICP0 protein must be present to interact with ICP27 for repression to occur.

DISCUSSION

To investigate the specificity of action of the HSV-1 α proteins ICP4, ICP0, and ICP27, we performed a series of transfection experiments in which plasmids containing each of these genes were transfected either singly or in combination with each other with a battery of HSV-1 and heterologous target plasmids. These experiments showed that ICP4 had specificity for HSV-1 promoters, whereas ICP0 was capable of inducing the SV40 early promoter or the RSV long terminal repeat as efficiently as HSV-1 promoters, consistent with the results of O'Hare and Hayward (70). In accord with previous findings, ICP27 on its own had little effect on HSV-1 promoters (11, 17, 25), nor did it have a substantial effect on heterologous promoters. The most interesting finding of these studies was that when a plasmid encoding ICP27 was added to transfections in combination with plasmids encoding ICP4 or ICP0 or both, a significant *trans*-repression or *trans*-activation of target gene activity occurred. Whether repression or activation occurred depended on the target promoter.

Several possible methods of action for ICP27 can be postulated from these results. Considering the *trans*-repressor activity, down-regulation of ICP0 mRNA levels by ICP27 (Fig. 4) suggests that ICP27 could be acting specifically on the promoter-regulatory region of the ICP0 gene, resulting in repression, similar to the autoregulatory activity of ICP4 (11, 24, 25, 65, 69, 70). The consequent lower level of ICP0 expression could result in decreased induction of target plasmids. This is unlikely to be the sole explanation for the *trans*-repressive effect of ICP27 observed, however, because even very low levels of input ICP0 plasmid (Table 4; Fig. 5) or low levels of ICP0 mRNA (Fig. 4) resulted in significant induction of target plasmids. Furthermore, the extent of down-regulation of ICP0 mRNA levels varied in different experiments, from little discernible effect (Fig. 3) to a significant effect (Fig. 4B), yet down-regulation of CAT expression was seen in all cases. In addition, ICP27 does not appear to show specificity for the ICP0 promoter, because when the ICP27 plasmid pSG130 B/S was added over a 10-fold concentration range to transfections with pICP0-CAT as the target, no significant effect on CAT expression was seen (Table 4). A similar result was found with pICP4-CAT, which contains the promoter from the ICP4 gene fused to CAT (data not shown). Furthermore, some down-regulation of other targets was seen with ICP27, although the effect was small, indicating that ICP27 does not specifically recognize the regulatory regions of ICP0 and ICP4. For example, down-regulation of CAT activity by ICP27 alone was seen with pICP27-CAT, pTK-CAT, and pAE-CAT (Table 4), and approximately threefold-lower levels of CAT mRNA were seen in the presence of ICP27 with pSV2-CAT as a target (Fig. 3). Finally, in transfections with pAE-CAT or pVP5-CAT as targets, a stimulation of CAT activity occurred beyond that seen with ICP4 or ICP0 as the only effector. Therefore, if ICP27 acted specifically to repress ICP4 and ICP0 expression, resulting in lower induction of target activity, it would be expected that this repression would occur regardless of the target, yet stimulation of CAT activity was seen with pAE-CAT and pVP5-CAT in the presence of ICP27, ICP4, and ICP0. For these reasons, other modes of action for ICP27 besides specificity for ICP4 or ICP0 promoter sequences should be considered.

During infection, ICP27 is clearly involved in the switch from early to late gene expression, because at the nonpermissive temperature, ICP27 temperature-sensitive mutants overproduce some immediate early and early gene products, whereas late gene products are underproduced (79; I. L. Smith and R. M. Sandri-Goldin, unpublished observations). The two activities of ICP27 found in the studies reported here, namely, a repressor function and an activator function, are consistent with the in vivo role of ICP27. Furthermore, these two activities involve different regions of the ICP27 protein. We have constructed and analyzed a series ICP27 mutants containing in-frame insertions or deletions. Several mutants have been identified which cannot activate pVP5-CAT in the presence of ICP4 and ICP0 but which can still repress pTK-CAT activity. Other mutants have been found which fail to repress pTK-CAT (M. A. Hardwicke, P. J. Vaughan, R. E. Sekulovich, B. O'Conner, and R. M. Sandri-Goldin, submitted for publication). ICP27 could perform these different functions in a number of ways. First, it could bind to specific regulatory sequences and thus prevent progression of the transcription complex, resulting in repression. The recognized sequences could be regulatory sequences like SP1 sites (13, 42) or CAAT boxes (60, 62) found on HSV-1 immediate early and early genes, as well as heterologous genes, but not on HSV-1 γ genes (36, 41, 83). Second, ICP27 could act by protein-protein interactions with ICP4 and ICP0, altering their specificities or modifying them in some way. Third, ICP27 could interact with some cellular transcription factor, altering or modifying it. Finally, a combination of mechanisms may occur such that ICP27 could bind to DNA in its role as a repressor and could interact with ICP4 and ICP0 to facilitate activation.

Although the data reported here suggest that ICP27 interacts with ICP4 and ICP0, biochemical evidence must be obtained to substantiate this hypothesis. Furthermore, studies must be performed to analyze the specificity of the ICP27 repressor function versus the activation function. In these experiments, the β-γ target pVP5-CAT was activated, whereas the β target pTK-CAT was repressed. These results are consistent with a proposed in vivo role for ICP27 in the shutoff of early functions and the activation of late functions. However, the β-γ targets pgD-CAT and pgB-CAT and the γ target pgC-CAT were also repressed, whereas the β target pAE-CAT was activated. A possible explanation for these findings is that these target genes contain different extents of upstream sequences. For example, the pgC-CAT construct contains approximately 1,100 bp upstream of the gC transcriptional start site, whereas only about 35 bp upstream from the cap site is necessary for full expression as a γ gene (83). Gelman and Silverstein (25, 26) and Blair and Wagner (2) have demonstrated that different lengths of upstream sequences have significant effects on the response of target genes to *trans*-acting factors. Therefore, it is necessary to identify the core sequences which are responding in each of these target plasmids. We have begun to do this with the VP5 and gC promoters. A nested set of promoter deletions are being analyzed for their ability to respond to ICP27.

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LITERATURE CITED

1. **Beard, P., S. Faber, K. W. Wilcox, and L. I. Pizer.** 1986. Herpes simplex virus immediate early infected-cell polypeptide 4 binds to DNA and promotes transcription. Proc. Natl. Acad. Sci. USA **83**:4016–4020.
2. **Blair, E. D., and E. K. Wagner.** 1986. A single regulatory region modulates both *cis* activation and *trans* activation of the herpes simplex virus VP5 promoter in transient-expression assays in vivo. J. Virol. **60**:460–469.
3. **Borelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. Nature (London) **312**:608–612.
4. **Brady, J., and G. Khoury.** 1985. *trans* activation of the simian virus 40 late transcription unit by T antigen. Mol. Cell. Biol. **5**: 1391–1399.
5. **Bzik, D. J., B. A. Fox, N. A. DeLuca, and S. Person.** 1984. Nucleotide sequence specifying the glycoprotein gene gB of herpes simplex virus type 1. Virology **133**:301–314.
6. **Carthew, R. W., L. A. Chodosh, and P. A. Sharp.** 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. Cell **43**:439–448.
7. **Cathala, G., G. F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial, and J. D. Baxter.** 1983. A method for the isolation of intact, translationally active ribonucleic acid. DNA **2**:329–335.
8. **Cordingley, M. G., M. E. M. Campbell, and C. M. Preston.** 1983. Functional analysis of a herpes simplex virus type 1 promoter: identification of far-upstream regulatory sequences. Nucleic Acids Res. **11**:2347–2365.
9. **Costa, R. H., K. G. Draper, G. Devi-Rao, R. L. Thompson, and E. K. Wagner.** 1985. Virus-induced modification of the host cell is required for expression of the bacterial chloramphenicol acetyltransferase gene controlled by a late herpes simplex virus promoter (VP5). J. Virol. **56**:19–30.
10. **DeLuca, N. A., A. M. McCarthy, and P. A. Schaffer.** 1985. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate-early regulatory protein ICP4. J. Virol. **56**:558–570.
11. **DeLuca, N. A., and P. A. Schaffer.** 1985. Activation of immediate-early, early, and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. Mol. Cell. Biol. **5**:1997–2008.
12. **Dixon, R. A. F., and P. A. Schaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. **36**:186–203.
13. **Dynan, W. S., and R. Tjian.** 1983. The promoter-specific transcription factor SP1 binds to upstream sequences in the SV40 early promoter. Cell **35**:79–87.
14. **Dynan, W. S., and R. Tjian.** 1983. Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. Cell **32**:669–680.
15. **Everett, R. D.** 1984. Trans-activation of transcription by herpes virus gene products: requirement for two HSV-1 immediate-early polypeptides. EMBO J. **3**:3135–3141.
16. **Everett, R. D.** 1984. A detailed analysis of an HSV-1 early promoter: sequences involved in trans-activation by viral immediate-early gene products are not early gene specific. Nucleic Acids Res. **12**:3037–3056.
17. **Everett, R. D.** 1986. The products of herpes simplex virus type 1 (HSV-1) immediate early genes 1, 2 and 3 can activate HSV-1 gene expression in trans. J. Gen. Virol. **67**:2507–2513.
18. **Everett, R. D.** 1986. A detailed mutational analysis of Vmw 110, a trans-acting transcriptional activator encoded by herpes simplex virus type 1. EMBO J. **6**:2069–2076.
19. **Everett, R. D., and M. Dunlop.** 1984. Trans-activation of plasmid-borne promoters by adenovirus and several herpes group viruses. Nucleic Acids Res. **12**:5969–5978.
20. **Faber, S. W., and K. W. Wilcox.** 1986. Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences in DNA. Nucleic Acids Res. **14**:6067–6083.
21. **Faber, S. W., and K. W. Wilcox.** 1988. Association of herpes simplex virus regulatory protein ICP4 with sequences spanning the ICP4 gene transcription initiation site. Nucleic Acids Res. **16**:555–570.
22. **Feldman, L. T., L. J. Imperiale, and J. R. Nevins.** 1982. Activation of early adenovirus transcription by the herpesvirus immediate-early gene: evidence for a common cellular control factor. Proc. Natl. Acad. Sci. USA **79**:4952–4956.
23. **Gelman, I. H., and S. Silverstein.** 1985. Identification of immediate-early genes from herpes simplex virus that can trans activate the virus thymidine kinase gene. Proc. Natl. Acad. Sci. USA **82**:5265–5269.
24. **Gelman, I. H., and S. Silverstein.** 1986. Co-ordinate regulation of herpes simplex virus gene expression is mediated by the functional interaction of two immediate early gene products. J. Mol. Biol. **191**:395–409.
25. **Gelman, I. H., and S. Silverstein.** 1987. Herpes simplex virus immediate early promoters are responsive to virus and cell *trans*-acting factors. J. Virol. **61**:2286–2296.
26. **Gelman, I. H., and S. Silverstein.** 1987. Dissection of immediate early gene promoters from herpes simplex virus: sequences that respond to the virus transcriptional activators. J. Virol. **61**: 3167–3172.
27. **Gilardi, P., and M. Perricaudet.** 1984. The Eγ transcriptional unit of Ad 2: far upstream sequences are required for its transactivation by E1A. Nucleic Acids Res. **12**:7877–7888.
28. **Goldin, A. L., R. M. Sandri-Goldin, M. Levine, and J. C. Glorioso.** 1981. Cloning of herpes simplex type 1 sequences representing the whole genome. J. Virol. **38**:50–58.
29. **Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79**:6777–6781.
30. **Gorman, C. M., L. L. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
31. **Green, M. R., R. Treisman, and T. Maniatis.** 1983. Transcriptional activation of cloned human β-globin genes by viral immediate-early gene products. Cell **35**:137–148.
32. **Hanahan, D.** 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166**:557–580.
33. **Hansen, U., D. G. Tenen, D. M. Livingston, and P. A. Sharp.** 1981. T antigen repression of SV40 early transcription from two promoters. Cell **27**:603–612.
34. **Hearing, P., and T. Shenk.** 1985. Sequence-independent autoregulation of the adenovirus type 5 E1A transcription unit. Mol. Cell. Biol. **5**:3214–3221.
35. **Hen, R., E. Borelli, C. Fromental, P. Sassone-Corsi, and P. Chambon.** 1986. A mutated polyoma virus enhancer which is active in undifferentiated embryonal carcinoma cells is not repressed by adenovirus-2 E1A products. Nature (London) **312**: 249–251.
36. **Homa, F. L., T. M. Otal, J. C. Glorioso, and M. Levine.** 1986. Transcriptional control signals of a herpes virus type 1 late (γ-2) gene lie within bases −34 to +124 relative to the 5′ terminus of the RNA. Mol. Cell. Biol. **6**:3652–3666.
37. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **41**:8–19.
38. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral peptides. Proc. Natl. Acad. Sci. USA **72**:1276–1280.
39. **Imperiale, M. J., L. T. Feldman, and J. R. Nevins.** 1983.

Activation of gene expression by adenovirus and herpesvirus regulatory genes acting in trans and by a cis-acting adenovirus enhancer element. Cell **35**:127–136.

40. **Imperiale, M. J., R. P. Hart, and J. R. Nevins.** 1985. An enhancer-like element in the adenovirus E2 promoter contains sequences essential for uninduced and E1A-induced transcription. Proc. Natl. Acad. Sci. USA **82**:381–385.
41. **Johnson, P. A., and R. D. Everett.** 1986. DNA replication is required for abundant expression of a plasmid borne late US11 gene of herpes simplex virus type 1. Nucleic Acids Res. **14**: 3609–3625.
42. **Jones, K. A., K. R. Yamamoto, and R. Tjian.** 1985. Two distinct transcription factors bind to the HSV thymidine kinase promoter *in vitro*. Cell **42**:559–572.
43. **Kao, H. T., and J. R. Nevins.** 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. Mol. Cell. Biol. **3**:2058–2064.
44. **Keller, J. M., and J. C. Alwine.** 1984. Activation of the SV40 late promoter: direct effects of T antigen in the absence of DNA replication. Cell **36**:381–389.
45. **Keller, J. M., and J. C. Alwine.** 1985. Analysis of an activatable promoter: sequences in the simian virus 40 late promoter required for T-antigen-mediated *trans*-activation. Mol. Cell. Biol. **5**:1859–1869.
46. **Khoury, G., and E. May.** 1977. Regulation of early and late simian virus 40 transcription: overproduction of early viral RNA in the absence of a functional T antigen. J. Virol. **78**:377–389.
47. **Kingston, R. E., R. J. Kaufman, and P. A. Sharp.** 1984. Regulation of transcription of the adenovirus EII promoter by E1A gene products: absence of sequence specificity. Mol. Cell. Biol. **4**:1970–1977.
48. **Kovesdi, I., R. Reichel, and J. R. Nevins.** 1986. Identification of a cellular transcription factor involved in E1A trans-activation. Cell **45**:219–228.
49. **Kovesdi, I., R. Reichel, and J. R. Nevins.** 1986. E1A transcription induction: enhanced binding of a factor to upstream promoter sequences. Science **231**:719–722.
50. **Kristie, T. M., and B. Roizman.** 1986. α 4, the major regulatory protein of herpes simplex virus type 1, is stably and specifically associated with the promoter-regulatory domains of α genes and selected other viral genes. Proc. Natl. Acad. Sci. USA **83**:3218–3222.
51. **Kristie, T. M., and B. Roizman.** 1986. Site of major regulatory protein α 4 specifically associated with promoter-regulatory domains of α genes of herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA **83**:4700–4704.
52. **Leff, T., J. Corden, and R. Elkaim.** 1985. Transcriptional analysis of the adenovirus 5 EIII promoter: absence of sequence specificity for stimulation by E1A gene products. Nucleic Acids Res. **13**:1209–1221.
53. **Leong, K., and A. J. Berk.** 1986. Adenovirus early region 1A protein increases the number of template molecules transcribed in cell-free extracts. Proc. Natl. Acad. Sci. USA **83**:5844–5848.
54. **Longnecker, R., and B. Roizman.** 1986. Generation of an inverting herpes simplex virus 1 mutant lacking the L-S junction a sequences, an origin of DNA synthesis, and glycoprotein E and the α 47 gene. J. Virol. **58**:583–591.
55. **Mackem, S., and B. Roizman.** 1982. Differentiation between α promoter and regulator regions of herpes simplex virus 1: the functional domains and sequence of a movable α regulator. Proc. Natl. Acad. Sci. USA **79**:4917–4921.
56. **Mackem, S., and B. Roizman.** 1982. Structural features of the herpes simplex virus a α 4, 0, and 27 promoter-regulatory sequences which confer α regulation on chimeric thymidine kinase genes. J. Virol. **44**:939–949.
57. **Mavromara-Nazos, P., M. Ackerman, and B. Roizman.** 1986. Construction and properties of a viable herpes simplex virus 1 recombinant lacking coding sequences of the α 47 gene. J. Virol. **60**:807–812.
58. **Mavromara-Nazos, P., S. Silver, J. Hubenthal-Voss, J. L. C. McKnight, and B. Roizman.** 1986. Regulation of herpes simplex virus 1 genes: α gene sequence requirements for transient induction of indicator genes regulated by β or late (γ-2) promoters. Virology **149**:152–164.
59. **McKnight, S., and R. Tjian.** 1986. Transcriptional selectivity of viral genes in mammalian cells. Cell **46**:795–805.
60. **McKnight, S. L.** 1982. Functional relationships between transcriptional control signals of the thymidine kinase gene of herpes simplex virus. Cell **31**:355–365.
61. **McKnight, S. L., E. R. Gavis, R. Kingsbury, and R. Axel.** 1981. Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. Cell **25**:385–398.
62. **McKnight, S. L., R. C. Kingsbury, A. Spence, and M. Smith.** 1984. The distal transcription signals of the herpes virus tk gene share a common hexanucleotide control sequence. Cell **37**:253–262.
63. **McMaster, G. K., and G. C. Carmichael.** 1977. Analysis of double- and single-stranded nucleic acids on polyacrylamide and agarose gels using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4839.
64. **Michael, N., D. Spector, P. Mavromara-Nazos, T. Kristie, and B. Roizman.** 1988. The DNA-binding properties of the major regulatory protein α 4 of herpes simplex viruses. Science **239**:1531–1534.
65. **Muller, M. T.** 1987. Binding of the herpes simplex virus immediate-early gene product ICP4 to its own transcriptional start site. J. Virol. **61**:858–865.
66. **Murthy, S. C. S., G. P. Bhat, and B. Thimmappaya.** 1985. Adenovirus E2 early promoter: transcription control elements and induction by the viral pre-early E1A gene, which appears to be sequence independent. Proc. Natl. Acad. Sci. USA **82**:2230–2234.
67. **Myers, R. M., D. C. Rio, A. K. Robbins, and R. Tjian.** 1981. SV40 gene expression is modulated by the cooperative binding of T antigen to DNA. Cell **25**:373–384.
68. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53**:751–760.
69. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56**:723–733.
70. **O'Hare, P., and G. S. Hayward.** 1987. Comparison of upstream sequence requirements for positive and negative regulation of a herpes simplex virus immediate-early gene by three virus-encoded *trans*-acting factors. J. Virol. **61**:190–199.
71. **Pellett, P. E., K. G. Kousoulas, L. Pereira, and B. Roizman.** 1985. Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. J. Virol. **53**:243–253.
72. **Perry, L. J., F. J. Rixon, R. D. Everett, M. C. Frame, and D. J. McGeoch.** 1986. Characterization of the IE110 gene of herpes simplex virus type 1. J. Gen. Virol. **67**:2365–2380.
73. **Pizer, L. I., D. G. Tedder, J. L. Betz, K. W. Wilcox, and P. Beard.** 1986. Regulation of transcription in vitro from herpes simplex virus genes. J. Virol. **60**:950–959.
74. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant ts K. J. Virol. **29**:275–284.
75. **Preston, V. G.** 1981. Fine-structure mapping of herpes simplex virus type 1 temperature-sensitive mutations within the short repeat region of the genome. J. Virol. **39**:150–161.
76. **Quinlan, M. P., and D. M. Knipe.** 1985. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. Mol. Cell. Biol. **5**:957–963.
77. **Reed, S. I., G. R. Stark, and J. C. Alwine.** 1976. Autoregulation of simian virus 40 gene A by T antigen. Proc. Natl. Acad. Sci. USA **73**:3083–3087.
78. **Rio, D. C., and R. Tjian.** 1983. T antigen binding site mutations that affect autoregulation. Cell **32**:1227–1240.
79. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.

80. **Sandri-Goldin, R. M., A. L. Goldin, L. E. Holland, J. C. Glorioso, and M. Levine.** 1983. Expression of herpes simplex virus β and γ genes integrated in mammalian cells and their induction by an α gene product. Mol. Cell. Biol. **3**:2028–2044.
81. **Sawadogo, M., and R. G. Roeder.** 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell **43**:165–175.
82. **Sears, A. E., I. W. Halliburton, B. Meignier, S. Silver, and B. Roizman.** 1985. Herpes simplex virus 1 mutant deleted in the α 22 gene: growth and gene expression in permissive and restrictive cells and establishment of latency in mice. J. Virol. **55**:338–346.
83. **Shapira, M., F. L. Homa, J. C. Glorioso, and M. Levine.** 1987. Regulation of the herpes simplex virus type 1 late (γ2) glycoprotein C gene: sequences between base pairs −34 to +29 control transient expression and responsiveness to transactivation by the products of the IE 4 and 0 genes. Nucleic Acids Res. **15**:3097–3111.
84. **SivaRaman, L., S. Subramanian, and B. Thimmappaya.** 1986. Identification of a factor in HeLa cells specific for upstream transcriptional control sequence of an E1A-inducible adenovirus promoter and its relative abundance in infected and uninfected cells. Proc. Natl. Acad. Sci. USA **83**:5914–5918.
85. **Straney, S. B., and D. M. Crothers.** 1987. Lac repressor is a transient gene-activating protein. Cell **51**:699–707.
86. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
87. **Tjian, R.** 1981. T antigen binding and control of SV40 gene expression. Cell **26**:1–2.
88. **Velcich, A., F. G. Kern, C. Basilico, and E. B. Ziff.** 1986. Adenovirus E1A proteins repress expression from polyomavirus early and late promoters. Mol. Cell. Biol. **6**:4019–4025.
89. **Velcich, A., and E. Ziff.** 1985. Adenovirus E1A proteins repress transcription from the SV40 early promoter. Cell **40**:705–716.
90. **Watson, R. J., and J. B. Clements.** 1978. Characterization of transcription-deficient temperature-sensitive mutants of herpes simplex virus type 1. Virology **91**:364–379.
91. **Watson, R. J., and J. B. Clements.** 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature (London) **285**:329–330.
92. **Watson, R. J., J. H. Weis, J. S. Salstrom, and L. W. Enquist.** 1982. Herpes simplex virus type 1 glycoprotein D gene: nucleotide sequence and expression in Escherichia coli. Science **218**: 381–384.