Regulation of the herpes simplex virus type 1 late (γ_2) glycoprotein C gene: sequences between base pairs -34 to +29 control transient expression and responsiveness to transactivation by the products of the immediate early (α) 4 and 0 genes

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ABSTRACT

The glycoprotein C (gC) gene of herpes simplex virus type 1 is a true late gene, in that its expression occurs late in infection with a strict requirement for viral DNA replication. Recently, we reported on gC expression during infection with mutant viruses carrying deletions in the gC gene promoter. Analysis of RNA extracted from cells infected with individual mutants showed that the DNA sequences required for regulated expression of this late gene lie within bases -34 to +124 relative to the 5' end of the mRNA. In the present study, the deleted gC promoter sequences were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene and expression was measured in short-term transfection assays after transactivation by infection with HSV or cotransfections with a second plasmid carrying the immediate early genes of HSV-1. The 63 base pair sequence located between -34 to +29 on the gC promoter was sufficient to give induction of CAT activity following infection and on cotransfection with plasmids which code for the immediate early gene products ICP4 and ICP0. This 63 base pair region contains the TATA homology and the transcriptional start site of the gC gene, and apparently contains specific promoter elements not found in a similar region of the HSV TK promoter. This was shown by removing the distal upstream region of the TK promoter, 5' to -37, and found that the TK gene was no longer activated by infection or cotransfection with an $\alpha 4-\alpha 0$ gene containing plasmid.

INTRODUCTION

Herpes simplex virus (HSV) is a large enveloped double stranded DNA virus. Its genome codes for approximately 80 genes, which are sequentially expressed during the lytic infectious cycle (1-3). The genes form at least three groups, the expression of which are tightly regulated at the level of transcription (4). The first group which is expressed immediately after infection, in the absence of de novo viral protein synthesis, consists of five genes, $\alpha 0$, $\alpha 4$, $\alpha 22$, $\alpha 27$ and $\alpha 47$. Transcription of these immediate early (IE or α) genes is enhanced in response to a component present in infecting virions, ICP16 (5,6). Functional ICP4 is required throughout infection for expression of early (β) and late (γ) genes (2,7). Functional ICP27 is required for late gene expression (8). Mutants defective in ICP27 allow the

expression of early but not late genes. The function carried out by ICP22 also involves late gene expression, but is only required in some cell lines and not in others (9). Late genes form two subclasses which differ with respect to their dependence on viral DNA synthesis. The expression of γ_1 genes is significantly reduced in the absence of DNA replication, whereas, γ_2 gene expression is completely inhibited.

Our laboratory has reported on a study to define the cis-acting sequences required for regulated expression of the gC gene, a model γ_2 gene of HSV-1 (10). Mutant viruses were isolated with variable 5' and 3' deletions of DNA within base pairs -569 to +124 relative to the site of initiation of the gC message. Analysis of RNA transcribed in cells infected with individual mutants showed that the DNA sequences required for expression of this γ_2 gene lie within base pairs -34 to +124. This 158 bp segment was sufficient to confer accurate and quantitative expression of gC mRNA and to maintain the stringent requirement for viral DNA replication for expression of this gene. Substitution of this 158 bp segment for the normal β thymidine kinase (TK) gene promoter/regulatory sequences in the TK locus, resulted in the transcription of this chimeric gene as a γ_2 gene in that TK expression in infected cells was dependent on viral DNA synthesis. More recent analysis has refined the gC gene regulatory region to a 63 bp sequence between -34 to +29 as sufficient for stringent γ_2 expression (Homa, Glorioso and Levine, in preparation). The only recognizable consensus sequence upstream of the transcription initiation site for the gC gene is the TATA sequence at -30. Sequences upstream of the TATA are not required for regulated expression of the γ_2 gC gene.

In this paper we examine the cis- and trans-acting factors required for expression of the gC gene in transient expression assays. The cis-acting sequences sufficient to give induced expression from a plasmid borne gC promoter were identical to those required in infection. Nucleotides -34 to +29 function as a good promoter in response to infection with wild type virus and on cotransfection with plasmids carrying both the α_4 and α_0 genes.

MATERIALS AND METHODS

Cell and virus

LTK⁻ cells were grown and maintained in Eagle minimum essential medium containing Hank's salts, supplemented with tryptose phosphate broth, nonessential amino acids, 10% heat inactivated fetal calf serum, 100 μ g/ml of streptomycin and 100 U/ml of penicillin. HSV-1 strain KOS (KOS 321) was propagated and titered on Vero cells as described (11).

Plasmid constructions

Hybrid genes of gC promoter and CAT coding sequences. pGC (Fig. 1), as described in detail in a previous publication (10), contained a 4.0 kb SalI-HindIII fragment (map units 0.620-0.647) of HSV-1 DNA cloned into pUC18. This fragment contains the entire 2.7 kb gC gene plus 1.3 kb of 5' flanking sequence. A series of plasmids containing deletions in the promoter sequences of the gC gene, which range between sequences -569 and +124 relative to the site of initiation of transcription, was previously prepared (10). The following plasmids were utilized: pGCA3 deleted for sequences -146 to +124; pGCA5 from -569 to -151; pGCA8, from -144 to -35; and pGCA9 from -144 to +14. Details on the construction of the deletion plasmids are described in detail elsewhere (10). A double deletion plasmid, pGCA10, was prepared in which sequences from +30 to +124 were deleted in addition to those from -144 to -35. This deletion plasmid was constructed in the following manner. pGCA8 was digested with HaeIII, which cuts at +29, and BglII linkers were inserted at that position. The plasmid was then digested with XhoI and BglII and the resulting 63 base pair fragment between -34 to +29 was gel purified and cloned into the pGCA8 vector, already carrying the -144 to -35 deletion, predigested with XhoI and BglII. Plasmids pGCA5, pGCA8 and pGCA10 all contained the TATA sequence homology and the transcriptional start site of the gC gene, whereas, the deletions in pGCA3 and pGCA9 removed these two elements.

In constructing the plasmid, pGC-CAT (Fig. 1), the coding region for the CAT enzyme was placed under the control of the wild type regulatory region of glycoprotein C gene of HSV-1. The HindIII and BamHI sites of pSVOCAT (provided by B. Howard) (12), which encompass the CAT gene coding sequence, were converted into BglII and HindIII sites respectively, using the appropriate linkers, resulting in plasmid pSVOCAT-BH. The 1.6 kb BglII-HindIII fragment of pSVOCAT-BH was then inserted into the BglII and HindIII sites of pGC, so that the CAT coding region replaced that of glycoprotein C, and was therefore under the control of the regulatory sequences of HSV-1 gC. Similarly, the CAT coding sequences were introduced into the gC deletion plasmids mentioned above, replacing the gC coding sequences (Fig. 1), resulting in plasmids pGCA3-CAT, pGCA5-CAT, pGCA8-CAT, pGCA9-CAT and pGCA10-CAT. In addition, for each of the plasmids shown in Fig. 1, a parallel series of the gC deletion plasmids was prepared, in which all the HSV-1 sequences 5' to the deletions were removed, by elimination of the region between the XhoI and SalI sites.

Hybrid genes of TK promoter sequences and CAT coding region. pTK-CAT (obtained from P. Schaffer) contained an 800 bp BamHI-BglII region of the TK promoter/

regulatory region 5' to the CAT coding sequence (13). pΔ37TK-CAT is a plasmid with an analogous deletion in the TK promoter to that found in the gC promoter of pGCA8-CAT, namely deletion of sequences which are 5' to the TATA box. This plasmid was prepared by inserting the BamHI-BglII fragment from S. McKnight's plasmid LS -47/-37(22) into the BglII site of pSVOCAT-BH in the correct orientation. The BamHI-BglII fragment contains the TK promoter sequences from -37 to +58 relative to the 5' end of the message. LS-47/-37 is a plasmid, in which a 10 bp BamHI linker replaces the original sequences of the TK promoter between positions -47 and -37. The TATA homology sequence in this plasmid is at position -25.

Plasmids containing IE genes. pSG1 contains the EcoRI-JK fragment of HSV-1 cloned in pBR325 (15). This fragment carries sequences which code for the IE genes ICP4 and ICP0. pSG28 contains the EcoRI EK fragment of HSV-1 and contains sequences which code for ICP4, ICP0 and ICP27 (15). Plasmid, pSG1-EK1, contains sequences which code for the ICP4 gene alone, was obtained from D. Knipe. This plasmid contained an EcoRI-KpnI fragment which was subcloned from pSG1 (16). pIGA 15 (obtained from S. Silverstein) contained sequences which code for ICP0 within an SstI-EcoRV fragment (17). pFH100 contained the ICP-22 gene, included in the BamHI N fragment of HSV-1 KOS cloned in pBR322. pSG25-SE11 contained the ICP47 gene, included in the EcoRI-SalI fragment which was subcloned from pSG25 (15). pSG130-KB (kindly provided by R. Sandri-Goldin) contained the ICP27 gene as a BamHI-KpnI fragment.

Transfections

LTK⁻ cells were plated one day before transfection at a density of 1.5×10^6 cells per dish (Nunc - 60 mm diameter). The transfections were performed according to the calcium phosphate precipitate method of Graham and van der Eb (18). Cells were overlaid with the DNA precipitate and incubated for 40 minutes at 37°C, at which time MEM containing 10% FCS was added. The cells were incubated for 3 additional hours at which time they were shocked with 15% glycerol in MEM for 2 minutes, and washed twice. Equimolar amounts of plasmid DNA were used in cotransfection experiments and the cells harvested 2 days after the transfection (40-44 hrs). When transactivation was attempted by viral infection, the transfected cells were infected one day after the transfection at an moi ranging from 0.5 to 3, and then harvested 12-16 hrs postinfection. Drugs used to prevent viral DNA replication were introduced one hour before virus adsorption and maintained throughout the infection, as described previously (10,19).

CAT assays

CAT assays were performed according to Gorman *et al* (12). The cells were washed three times with PBS and scraped from the dishes into Tris buffered saline which contained 2 mM EDTA, pelleted and resuspended in 100 μ l of 0.25M Tris:HCl pH 7.8. The cells were extracted by three cycles of freezing and thawing followed by a 15 min. spin in a microfuge at 4°C. The extracts were heated at 55°C for 5 min. and centrifuged again to remove nonspecific acetylases. The enzymatic reaction was carried out at 37°C and contained 0.2 μ Ci of [14 C] chloramphenicol (NEN/DuPont), 20 mM Acetyl CoA (Pharmacia) and 0.2 M Tris HCl pH 7.8. Upon termination, the reaction mixture was extracted with 1 ml of ethyl acetate. The organic phase was dried, resuspended in 20 μ l of ethyl acetate and spotted on silica gel thin layer chromatography plates and subjected to ascending chromatography in chloroform: methanol (95:5). The plates were autoradiographed on Kodak XAR5 film. Enzymatic activity was quantitated by scraping the radioactive spots on the silica gel plates and counting them in scintillation fluid (OPS, Amersham). Counts were normalized to protein content which was determined by the Bradford method (20) using Biorad reagent. When the results fell out of the linear range, the assay was repeated with a smaller amount of extract.

RESULTS

gC-CAT plasmid constructions.

To characterize the cis- and trans-acting elements which control expression from the gC promoter in transient assays, chimeric plasmids were constructed, in which the wild type gC promoter and deletion variants were fused to the coding sequences of an indicator gene, the chloramphenicol acetyltransferase (CAT) gene (Fig. 1). A 1.3 kb SalI-BglII fragment, containing the 5' transcribed noncoding and upstream sequences of the wild type gC gene, was fused at nucleotide +124 to the CAT structural sequences, to create plasmid pGC-CAT. In addition, plasmids were constructed which contained fusions between the CAT coding gene and several gC promoter deletions. pGCA5-CAT contained a deletion between nucleotides -569 to -151. pGCA8-CAT resulted from removal of sequences -144 to -35. pGCA10-CAT contained the same deletion as pGCA8-CAT and an additional deletion removing sequences between +30 to +124. These three constructs retain the gC TATA homology and mRNA start site. The deletions in pGCA3-CAT and pGCA9-CAT lack the TATA homology and the transcriptional start site by removal of the sequences between bases -146 to +124 and -144 to +14, respectively.

Figure 1. Detailed structure of pGC-CAT and pGC-CAT deletion plasmids. At the top, the HSV-1 genome is presented in the prototype arrangement showing the location of the 4kb SalI-HindIII fragment (map units 0.620-0.647) present in plasmid pGC. Plasmid pGC-CAT was derived from pGC by deleting the coding sequences of the gC gene from the BglII to the HindIII site followed by insertion of the coding sequences of the CAT gene. The CAT structural sequences are therefore fused to the gC promoter at base +124 (BglII site) relative to the 5' terminus of the gC mRNA. Plasmids pGCΔ3-CAT, pGCΔ5-CAT, pGCΔ8-CAT, pGCΔ9-CAT and pGCΔ10-CAT were constructed in a similar manner using pGC deletion plasmids previously described (10). The HSV sequences appear as labeled blocks, and the CAT sequences as empty blocks. The extent of the internal deletions carried by the pGC-CAT deletion plasmids are indicated by open areas between vertical lines, with the bases deleted from each plasmid shown above the open areas, +1 being the nucleotide found at the 5' terminus of the gC mRNA.

gC-CAT is induced by KOS infection

The responsiveness of the gC-CAT chimeric gene to induction was tested by transfection of pGC-CAT into LTK⁻ cells followed by infection with wild type virus. The infecting virus was expected to supply transacting factors necessary for induction of expression of the chimeric gene. CAT activity measured in extracts from these cells, showed an 8-9 fold increase above the low constitutive level of expression observed from transfection with pGC-CAT alone (Fig. 2). Expression of late genes during infection is dependent on the occurrence of DNA replication. However, when late genes reside outside of the viral genome, they are expressed independently of viral DNA synthesis (21). Expression of the gC-CAT gene in a transfection activated by infection was

Figure 2. CAT expression from cells transfected with pGC-CAT and infected with KOS in the presence and absence of drugs which inhibit DNA replication. LTK⁻ cells were transfected with 5 μ g of pGC-CAT and infected on the following day with wild type KOS at a multiplicity of 3 PFU/cell. At 16 hrs post infection, the cells were harvested, extracted and assayed for CAT activity. For inhibiting DNA replication, PAA at 30 μ g/ml and araT at 50 μ g/ml were present from 1 hr before infection until harvest. The results were normalized to the same protein content and expressed as percent of [14 C] chloramphenicol acetylated.

tested in the presence of drugs which inhibit viral DNA replication. Comparable levels of CAT activity were observed in the presence and absence of viral DNA replication (Fig. 2). Additional evidence for the independence from DNA synthesis of expression from the plasmid borne gC-CAT gene, comes from an experiment in which cells transfected with pGC-CAT were infected with the HSV-1 mutant ts16 at the nonpermissive temperature. This virus carries a temperature sensitive mutation in the gene for the viral DNA polymerase and does not show viral DNA synthesis at 39°C (22). Mutant infections at 39°C resulted in the absence of late gene expression. Cells transfected with pGC-CAT followed by infection with ts16 at the nonpermissive temperature produced high levels of CAT activity, 8 fold higher than in the absence of infection (data not shown).

gC promoter sequences between -34 to +29 are inducible by KOS infection.

To identify the cis-acting DNA sequences essential for induction of the wild type gC promoter, the gC-CAT deletion plasmids (Fig. 1) were transfected into LTK⁻ cells and then infected with wild type virus. The results are

Table 1. CAT expression from gC wild type and deletion promoter plasmids transactivated by KOS infection

KOS infection	Transfecting plasmids					
	pGC-CAT	pGCA5-CAT	pGCA8-CAT	pGCA10-CAT	pGCA3-CAT	pGCA9-CAT
-	4,242	3,152	2,470	6,127	5,413	4,178
+	27,988(6.6)	17,817(5.6)	12,133(4.9)	21,764(3.5)	4,053(0.7)	4,344(1.0)

Monolayers of LTK⁻ cells were transfected with 5 µg plasmid in duplicates. After 24 hrs the cells were infected with wild type KOS at a multiplicity of 3 PFU/cell. Sixteen hours postinfection, the cells were harvested, extracted, and assayed for protein and CAT activity. The results are presented as cpm of [¹⁴C]acetylated chloramphenicol, followed by the ratio of infected over non infected cells in parenthesis, and are normalized to the same protein content.

presented in Table 1. Induction of CAT expression was observed in cells transfected with pGC-CAT, pGCA5-CAT, pGCA8-CAT and pGCA10-CAT. The structural feature common to these plasmids was the gC gene TATA sequence at -30 and the transcriptional initiation site. In contrast, plasmids pGCA3-CAT and pGCA9-CAT did not show any elevation of CAT activity following infection. The deletions in these plasmids have removed the TATA sequence and the transcriptional start site. These results lead to the conclusion that the essential sequences for induction of expression from the gC promoter are present between bases -34 and +29 and that deletion of the region between -34 and +14 destroys the responsiveness to transactivation.

The gC promoter is transactivated by ICP4 and/or ICP0 in cotransfection

To examine the effect of immediate early gene products on the the activation of the gC promoter, the pGC-CAT plasmid was cotransfected with

Figure 3. CAT expression from cells cotransfected with pGC-CAT and plasmids which code for various immediate early genes. LTK⁻ cells were cotransfected with equimolar amounts of pGC-CAT and plasmids which code for the various immediate early genes. The cells were harvested at 40-44 hrs post-transfection, extracted and assayed for CAT activity.

Figure 4. CAT expression from cells cotransfected with pGC-CAT wild type or deletion plasmids, and with immediate early genes ICP4 and ICP0 coded for by plasmid pSG1. The experimental details are as described in the legend to Fig. 3. The results were quantitated, normalized to the same protein content and expressed as relative activity measured in the presence of transactivating factors over activity in the absence.

plasmids which code for each of the five immediate early genes (Fig. 3). Only cotransfections with the ICP4 and ICP0 genes induced CAT activity. Moreover, the combination of ICP4 and ICP0 genes gave higher induction than would be expected from additive effects alone. When the ICP27 gene was introduced together with the ICP4 and ICP0 genes, it had little additional effect on the level of CAT expression from pGC-CAT. No induction of CAT activity was detected in cotransfections with individual plasmids carrying the ICP22, ICP27 or ICP47 genes.

The region of the gC promoter which is responsive to ICP4 and ICP0 transactivation lies between base pairs -34 to +29.

To show that the cis-acting DNA sequences which are inducible by viral infection are also responsive to induction by ICP4 and ICP0, the gC-CAT deletion plasmids were cotransfected with plasmid pSG1, which codes for the ICP4 and ICP0 genes (Fig. 4). A similar sequence requirement was observed for transactivation by ICP4 and ICP0 as was found with infection (10). Plasmids pGC-CAT, pGCA5-CAT, pGCA8-CAT and pGCA10-CAT were responsive to induction by

cotransfection with ICP4 and ICP0. Plasmids pGCA3-CAT and pGCA9-CAT showed no elevation in CAT activity. These data narrow the sequences which are responsive to transactivation by the two α proteins to the region from -34 to +29.

Because pGCA5-CAT, pGCA8-CAT and pGCA10-CAT all retained HSV-1 sequences 5' of their respective deletions (See Fig. 1), the possibility remained that these upstream sequences play a role in regulating gC expression. These sequences were removed from the three plasmids by an XhoI-SalI digestion (Fig. 1), followed by ligation of the compatible ends to recircularize the vectors. Cotransfections with these plasmids and pSG1 showed CAT activity equivalent to that reported in Fig. 4 for the plasmid retaining the upstream sequences (data not shown). These findings support the conclusion that the sequences necessary and sufficient for induction of expression from the gC promoter are present between bases -34 to +29 relative to the start site of transcription.

Deletion of TK promoter sequences 5' to the TATA box abolishes expression from the TK promoter

To compare the characteristics of the gC regulatory region, we asked if the region of the early TK gene, analogous to -34 to +29 of the gC gene, has promoter activity. The TK promoter has been well characterized by McKnight and his colleagues (14,23,24) and shown to contain regions essential for TK expression stretching 110 bp upstream of the transcriptional start site. A plasmid was constructed in which sequences were deleted upstream of the TK gene TATA box. A 95 base pair fragment which extends from bases -37 to +58 as a BamHI-BglII fragment of the TK gene, was inserted, in the correct orientation, in front of the CAT coding sequence in pSVOCAT-BH. This resulted in plasmid p Δ 37TK-CAT in which the CAT coding region was under the control of the TK promoter whose 5' boundary extended just past the TATA box. This construction parallels that of pGC- Δ 8CAT. Two experiments were performed. In the first, LTK⁻ cells were transfected with p Δ 37TK-CAT and infected with KOS virus (Table 2). In the second, LTK⁻ cells were cotransfected with p Δ 37TK-CAT and pSG1 (Fig. 4B). No induction of CAT activity was observed in either case from p Δ 37TK-CAT, whereas high levels of CAT activity were induced in cells transfected with wild type pTK-CAT. These findings are in agreement with those of Eisenberg et al. (24) who showed that HSV-1 infection does not activate a plasmid borne TK gene carrying an identical deletion in its promoter region to that of p Δ 37TK-CAT. These data emphasize an inherent difference between the gC promoter and the TK promoter. The activity of the

Table 2. CAT expression from TK wild type and deletion promoter plasmids transactivated by KOS infection.

KOS infection	Transfecting Plasmids	
	PTK-CAT	pΔ37TK-CAT
-	3,496	1,842
+	13,375(3.8)	1,195(0.6)

For experimental details see Table 1

gC promoter relies only on the presence of the TATA box and sequences immediately downstream of it.

DISCUSSION

We have previously reported (10) on the analysis of the cis-acting sequences required for the regulated expression of the HSV-1 late (γ_2) gC gene during infection. In this paper we extend these studies to regulation of the gC promoter in transient expression assays. Plasmids were constructed fusing the wild type and deleted gC promoter sequences to an indicator gene, the CAT gene. Following transfection of these constructs into mouse L cells, induction of CAT activity was attempted by two methods: (i) infection with wild type virus to provide all the necessary trans-acting factors; and (ii) by cotransfection with a second plasmid carrying immediate early genes to define which positive regulatory factors are responsible for transactivation.

The same sequences are required for expression from the gC promoter in transfected plasmids as was demonstrated for expression from the viral genome. Plasmids carrying the gC TATA homology and the transcriptional initiation site responded to induction of CAT activity by infecting virus. Plasmids in which these two elements were deleted did not respond to infection. As was found for virus, the 63 bp from -34 to +29 are sufficient to give inducible CAT activity. Sequences upstream of the gC TATA homology play no apparent role in gC regulation.

The viral trans-acting regulatory factors involved in expression of the gC gene were identified with plasmids bearing the immediate-early genes of HSV-1. Plasmids carrying the genes for ICP4 and ICP0 each induced significant CAT activity from the wild type gC promoter. Cotransfection with a plasmid carrying the genes for both ICP4 and ICP0 resulted in higher levels of CAT activity than expected from that induced by each gene alone, suggesting a cooperative interaction between ICP4 and ICP0 in the regulation of the gC gene. Again, the 63 bp from -34 to +29 were found to be sufficient for

transactivation by ICP4 and ICP0. Plasmids deleted for the TATA homology and the start site for transcription of the gC gene failed to respond to transactivation by ICP4 and ICP0. Plasmids carrying the genes for ICP22, 27 or 47 failed to induce CAT activity.

Johnson and Everett (44), studying the cis-acting sequences of the HSV-1 late gene US11 in transient assays, concluded that promoter activity resides within sequences which include the TATA homology and the transcriptional start site and that no sequences upstream of these elements are required. This corroboration of our findings suggests that γ_2 genes are unique among HSV genes in that all signals necessary for fully regulated transcription lie in the TATA homology and sequences 3' to it and that sequences 5' to the TATA are not required. This is in sharp contrast to α and β genes. The regulated expression of both α and β genes is dependent on defined sequences upstream of the TATA homology. For α genes, promoter sequences are located approximately 110 bp 5' of the site of initiation of transcription and allow expression but not induction. The regulatory sequences are located upstream of -110 and confer α -specific induction (25-28). In the case of the early genes, TK and gD, sequences necessary for both expression and induction lie within 100 bp for TK, and 83 bp for gD, of the transcription start sites (14,23,24,29-34). In addition, the γ_1 gene VP5, requires sequences up to 125 bp 5' to the transcription start site for its activation by infection (35).

The gC regulatory region appears to be a unique moveable entity. When moved in front of the TK structural gene in the viral genome it functions as a γ_2 promoter. The gC-TK hybrid gene requires DNA synthesis for induction of TK during infection (10). The TATA sequence from the TK gene has none of the responsiveness to induction found for the gC sequence. Plasmids carrying residues -37 to +58 of the TK gene, placed in front of the CAT gene, lack promoter activity and fail to respond to transacting factors from infecting virus or from transfection with plasmids carrying the immediate early genes 4 and 0. This sequence contains the TK TATA homology and the site of initiation of transcription. These data suggest that not all promoter elements in this region are the same and interchangeable.

While there is a strict requirement for viral DNA synthesis for expression of γ_2 genes in infection, DNA synthesis by itself is not sufficient for expression. Expression of γ_2 genes is directly dependent on the positive regulatory activity of the α_4 protein (8,13,36). DeLuca et al. (36) have reported that infections with some α_4 ts mutants show the synthesis of early

proteins and viral DNA at nonpermissive temperature, yet late proteins are not detected. The $\alpha 4$ protein may have multiple domains, one of which is specific for γ gene activation. The transient assay data described here and by Mavromara-Nazos et al. (37) support a direct role for the $\alpha 4$ protein in $\gamma 2$ gene regulation. Kristie and Roizman (38) have recently reported that a labeled DNA fragment containing the promoter region of a $\gamma 2$ gene forms stable complexes with proteins from infected cell lysates as detected by a gel electrophoresis binding assay. A monoclonal antibody to the $\alpha 4$ protein retarded the mobility of the DNA-protein complex, indicating that the $\alpha 4$ protein forms a stable complex with late gene promoter sequences. The requirement for viral DNA synthesis is not manifested in transient assays, the $\alpha 4$ gene product alone is sufficient for gC induction. The role of viral DNA synthesis in the activation of the gC gene during infection remains obscure.

The role of the other α gene products in HSV gene regulation is as yet not understood. The α genes 22 and 47 appear not to be essential for virus production in some cell lines (9,39-41) and, as reported here, and by others, (37) do not induce late gene expression in transient assays. Induction of expression of β and γ genes in transient assay systems by the $\alpha 0$ gene product has been reported (39,42,43). We have confirmed these findings and a synergistic effect on gC activation with the combination of $\alpha 4$ and $\alpha 0$ genes. Yet, the $\alpha 0$ gene product can not substitute for the $\alpha 4$ gene in infections with $\alpha 4$ mutants. Infections with mutants mapping in the $\alpha 27$ gene fail to induce late gene expression (8), suggesting a role for this gene product in the regulation of late genes. No significant increase in CAT activity was detected in cotransfection of the gC-CAT plasmid with a plasmid carrying the $\alpha 27$ gene alone or in combination with the $\alpha 4$ and $\alpha 0$ genes. The inconsistencies between the results on infection and transfection remain unclear.

Our analysis of the sequences critical for expression of the gC gene continues. Studies designed to explore the interactions between these sequences and host and viral transcriptional factors are in progress.

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