JOURNAL OF VIROLOGY, Mar. 1989, p. 1232–1238
0022-538X/89/031232-07$02.00/0

Vol. 63, No. 3

Regulated Expression of the Human Cytomegalovirus pp65 Gene: Octamer Sequence in the Promoter Is Required for Activation by Viral Gene Products

ALISON S. DEPTO AND RICHARD M. STENBERG*

Department of Microbiology and Immunology, West Virginia University Health Sciences Center, Morgantown, West Virginia 26506

Received 8 September 1988/Accepted 5 December 1988

To better understand the regulation of late gene expression in human cytomegalovirus (CMV)-infected cells, we examined expression of the gene that codes for the 65-kilodalton lower-matrix phosphoprotein (pp65). Analysis of RNA isolated at 72 h from cells infected with CMV Towne or *ts*66, a DNA-negative temperature-sensitive mutant, supported the fact that pp65 is expressed at low levels prior to viral DNA replication but maximally expressed after the initiation of viral DNA replication. To investigate promoter activation in a transient expression assay, the pp65 promoter was cloned into the indicator plasmid containing the gene for chloramphenicol acetyltransferase (CAT). Transfection of the promoter-CAT construct and subsequent superinfection with CMV resulted in activation of the promoter at early times after infection. Cotransfection with plasmids capable of expressing immediate-early (IE) proteins demonstrated that the promoter was activated by IE proteins and that both IE regions 1 and 2 were necessary. Analysis of promoter deletion mutants indicated that the 5′ minimal sequence required for activation is −61 from the CAP site (+1) and that an 8-base-pair sequence located at −51 to −58 is necessary for activation of the pp65 promoter. This sequence is repeated once at +93 and is found as an inverted repeat at +67. These studies suggest that interactions between IE proteins and this octamer sequence may be important for the regulation and expression of this CMV gene.

Gene expression in human cytomegalovirus (CMV)-infected cells occurs in an ordered sequential fashion. CMV genes are divided into three groups based on their time of appearance in permissively infected cells (6, 7, 21, 42, 43). Immediate-early (IE) genes are the first genes to be transcribed after viral infection and require no de novo protein synthesis for their expression (6, 7, 21, 42, 43). IE gene products are required for subsequent expression and regulation of early genes (6, 7, 21, 42, 43) and may be required for late gene expression. Early gene expression occurs after IE protein synthesis but before viral DNA replication (6, 7, 21, 22, 42, 43). Early gene transcription proceeds in the presence of phosphonoacetic acid (PAA), an inhibitor of viral DNA polymerase, and therefore, early genes do not require viral DNA synthesis for expression. Late gene expression occurs after the initiation of viral DNA synthesis. Viral RNA at late times originates from all regions of the genome (6, 7, 21, 42, 43), and it is generally thought that the majority of late mRNAs codes for the structural components of the virus. It has been postulated that all CMV late genes are transcribed early but that late mRNA is not maximally translated until late times as a result of posttranscriptional controls (10). Also, it has been demonstrated that one late gene is transcribed early but that RNA does not appear in the cytoplasm until late (11). However, late genes requiring viral DNA replication for their maximal expression have been demonstrated (17, 24, 25), as well as true late genes that absolutely require DNA replication prior to expression (R. M. Stenberg et al., unpublished data). The mechanisms involved in late gene regulation have not been extensively studied, and it is unclear if or why there is a dependency on viral DNA replication for expression of some late genes.

* Corresponding author.

To gain insight into the mechanisms that play a role in the regulation of the 65-kilodalton lower-matrix phosphoprotein (pp65) gene (25, 26, 30), RNA expression and promoter activation were investigated. Although abundant RNA expression from the pp65 gene did not occur until after DNA replication, the pp65 promoter-CAT construct was activated very early under transient conditions. Analysis of promoter deletion mutants revealed that sequence 5′ to −61 could be removed with little effect on promoter activation and that an 8-nucleotide sequence, ATTTCGGG, may be important for the expression and regulation of this early-late gene.

MATERIALS AND METHODS

Virus and cells. The Towne strain of CMV was used exclusively for these studies. Propagation of virus in primary human foreskin fibroblast (HF) cells was performed as previously described (38). *ts*66 is a temperature-sensitive DNA-negative mutant of CMV Towne isolated from CMV-infected cells treated with 20 μg of nitrosoguanidine per ml. The virus was selected from a plaque that failed to enlarge at 39.5°C, is negative for viral DNA replication, and produces no detectable virus at the nonpermissive temperature (39.5°C) (Stenberg et al., unpublished data). The permissive temperature for *ts*66 is 33.5°C.

Enzymes. Enzymes were obtained from New England BioLabs, Inc. (Beverly, Mass.), Boehringer Mannheim Biochemicals (Indianapolis, Ind.), or Promega Biotec (Madison, Wis.) and were used as recommended by the manufacturer or as described elsewhere (35–37).

Plasmid DNAs. The plasmid pSVCC3 is derived from pSVCC2 which has been previously described (35). This plasmid is identical to pSVCC2 except that the *Bam*HI site in the pSVOd (23) sequences has been eliminated by *Bam*HI digestion, Klenow polymerase repair of the recessed 3′ end,

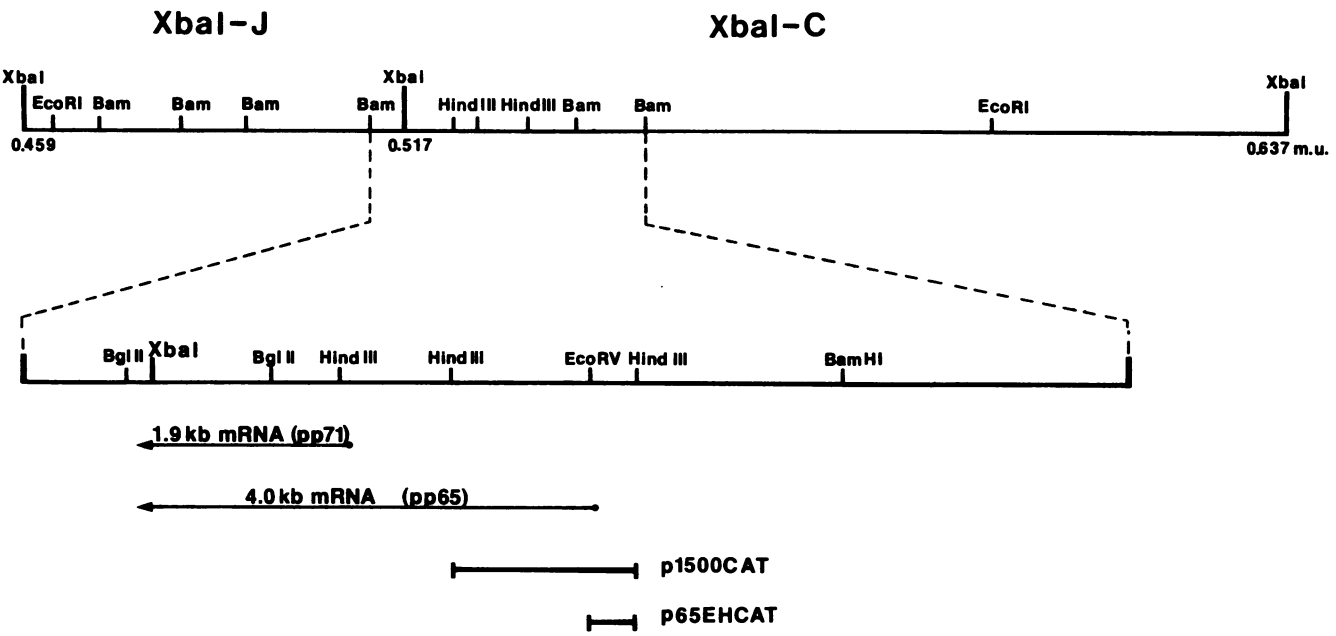

FIG. 1. Restriction endonuclease map of *Xba*I J and *Xba*I C. The 4-kb pp65 mRNA and 1.9-kb pp71 mRNA are shown below the expanded map. The 1.5-kb *Hin*dIII fragment of *Xba*I C was cloned into pSVOCATΔintron to produce p1500CAT. This 1.5-kb *Hin*dIII fragment was used to prepare an antisense RNA probe for pp65 RNA. p65EHCAT contains the *Eco*RV-to-*Hin*dIII fragment representing the promoter of the pp65 gene (−306 to +70). m.u., Map unit.

and blunt end ligation. The plasmid pSVH was constructed by inserting the *Bam*HI-to-*Sal*I fragment containing IE region 2 (39) into pSVCC3 from *Bam*HI to *Hin*dIII at the 3′ end of pSVCC3 (IE region 1). Prior to this step, the *Sal*I site was repaired with Klenow polymerase, *Hin*dIII linkers were added, and the resulting fragment was digested with *Hin*dIII and *Bam*HI. The *Hin*dIII linker chosen fully reconstructs the *Sal*I site. The plasmid pAcc was constructed by partial digestion of pSVH with *Acc*I, followed by selection of the desired fragment (which lacked the 1,414-nucleotide *Acc*I fragment containing exon 4 of IE 1 [36]) from a polyacrylamide gel and religating the product. Therefore, this deleted exon 4 of IE 1 from the final construct. The CMV *Xba*I C and *Xba*I J recombinant plasmids were obtained from Mark Stinski and have been described elsewhere (41). Subclones of the CMV *Xba*I plasmids and other CMV DNA sequences were cloned into the vectors pUC12, pGEM-3Z (Promega), or pSVOCATΔintron by standard techniques (19, 35–37). The plasmid pSVOCATΔintron was obtained from Jeannine Strobl. It is a derivative of pSV2CAT (12) containing a 507-base-pair (bp) deletion (positions 1 to 507 on the pSV2CAT map [12]) that removes the simian virus 40 promoter regulatory sequences and a 610-bp deletion (positions 3610 to 4220) (12) that eliminates the simian virus 40 small-t-antigen splice site.

Isolation and analysis of RNA. For all RNA expression studies, infected-cell RNA was obtained from HF cells infected with CMV at 10 to 20 PFU per cell. Whole-cell RNA was isolated by the method of Strohman et al. (40) as described previously (35, 37). Cytoplasmic RNA was prepared as described previously (3, 34). Early RNA was isolated from cells infected and maintained with or without 200 μg of the viral DNA polymerase inhibitor PAA per ml, and late RNA was isolated at 72 h after infection. Formaldehyde agarose gel electrophoresis and Northern (RNA) blot analysis was performed as previously described (19, 37).

Radiolabeling of DNA and RNA probes. CMV DNA sequences were radiolabeled to greater than 5×10^8 cpm/μg by the oligolabeling method of Feinberg and Vogelstein (9). DNA sequences to be used as templates for producing RNA probes were cloned into pGEM-3Z. pGHH1500 contains the 1,500 bp *Hin*dIII fragment of *Xba*I C (cloned from p1500CAT [Fig. 1]) inserted into pGEM-3Z in an orientation to permit synthesis of antisense RNA relative to pp65 mRNA. RNA probes were produced from linearized templates in transcription reactions containing 50 μCi of [α-^{32}P]CTP (400 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) and SP6 polymerase (Promega).

Promoter-CAT constructs. Construction of the pp65 promoter-CAT plasmid was as follows. A 1.5-kilobase (kb) *Hin*dIII fragment was isolated from *Xba*I C and cloned into pSVOCATΔintron, resulting in p1500CAT (Fig. 1). This plasmid was digested with *Eco*RV, followed by partial digestion with *Hin*dIII to remove 1.1 kb of 3′ sequence. The *Hin*dIII site was repaired with Klenow polymerase, and the DNA was blunt-end ligated to obtain p65EHCAT, which contains 376 bp of viral sequence (−306 to +70 relative to the CAP site [+1]). To produce pP65CAT and pP65CATrev, the 376-bp *Eco*RV-to-*Hin*dIII fragment of p1500CAT was isolated, the ends were repaired with Klenow polymerase, and *Hin*dIII linkers were added. This fragment was cloned into the *Hin*dIII site of pSVOCATΔintron, and plasmids containing the 376-bp promoter fragment in both orientations were isolated.

Deletions in the promoter region of p65EHCAT were constructed by restriction enzyme digestion and excessive mung bean nuclease digestion. The 5′ upstream sequences from −306 to −82 of p65EHCAT were removed by digestion with *Hin*dIII and *Sac*II (see Fig. 3). Plasmid DNA was then treated with 5 U of mung bean nuclease per μg of DNA for 1 h at 30°C and repaired with Klenow polymerase. *Hin*dIII linkers were ligated to the fragment, and the plasmid was recircularized, resulting in p65dl3CAT (−81 to +70). Other promoter deletion plasmids were constructed by linearize-

tion of p65dl3CAT or p65EHCAT with *Hin*dIII, followed by excessive mung bean nuclease treatment as described above, repair with Klenow polymerase, and subsequent recircularization. Sequencing of the pp65 promoter deletions was performed by standard dideoxy methods as described previously (31), except that Klenow polymerase reactions were incubated at 50°C.

Promoter activation. Promoter activation was tested by transfection of CAT plasmids, followed by superinfection with CMV or by cotransfection with various IE gene plasmids. Cultures of HF cells (85 to 90% confluent) were transfected with 5 μg of the indicated plasmid DNA, using DEAE dextran as described previously (35). Cells cotransfected with IE gene plasmids were harvested 48 h after transfection. To test activation by CMV, transfected cells were infected 18 h later at 10 to 20 PFU per cell. Cells were harvested at indicated times, and extracts were prepared and analyzed essentially as described by Gorman (12). To determine percent acetylation, each form of chloramphenicol was excised from the thin-layer chromatography plate and quantitated in a liquid scintillation counter. Assays were linear for at least 30 min for up to 50% acetylation.

RESULTS

Analysis of early and late gene expression. To better understand the regulation of pp65, a protein abundantly expressed late in CMV replication, HF cells were infected with CMV and whole-cell RNA was isolated at various times after infection. RNA from an equivalent number of infected cells was subjected to formaldehyde agarose gel electrophoresis and Northern blot analysis. Using a specific antisense RNA probe, low levels of pp65 RNA were detected as early as 5 h after infection and remained relatively constant through 24 h (Fig. 2A). At 48 h after infection, the level of pp65 RNA increased significantly, and maximum expression occurred at 72 h after infection. In the presence of PAA, the levels of pp65 RNA isolated at 24 and 72 h after infection were equivalent to levels detected at early times (5 to 24 h). These results demonstrate that the gene for pp65 is a member of the early-late class of genes that are expressed at low levels early and are greatly amplified after DNA replication. To further address and confirm this finding, we utilized a DNA-negative temperature-sensitive mutant of CMV (*ts*66). Cytoplasmic RNA was isolated at 72 h after infection at the nonpermissive temperature (39.5°C) from cells infected with *ts*66 or wild-type (WT) CMV. pp65 RNA was expressed in *ts*66-infected cells at levels approximately equal to those seen in WT-infected cells at early times and at significantly lower levels than those in WT-infected cells at late times (Fig. 2B). Analysis of whole-cell RNA isolated from *ts*66- or WT-infected cells at the nonpermissive temperature revealed the same pattern of expression as cytoplasmic RNA (data not shown). Therefore, the pp65 gene is an early-late gene which expresses low levels of RNA early, with maximal expression occurring after the initiation of DNA replication.

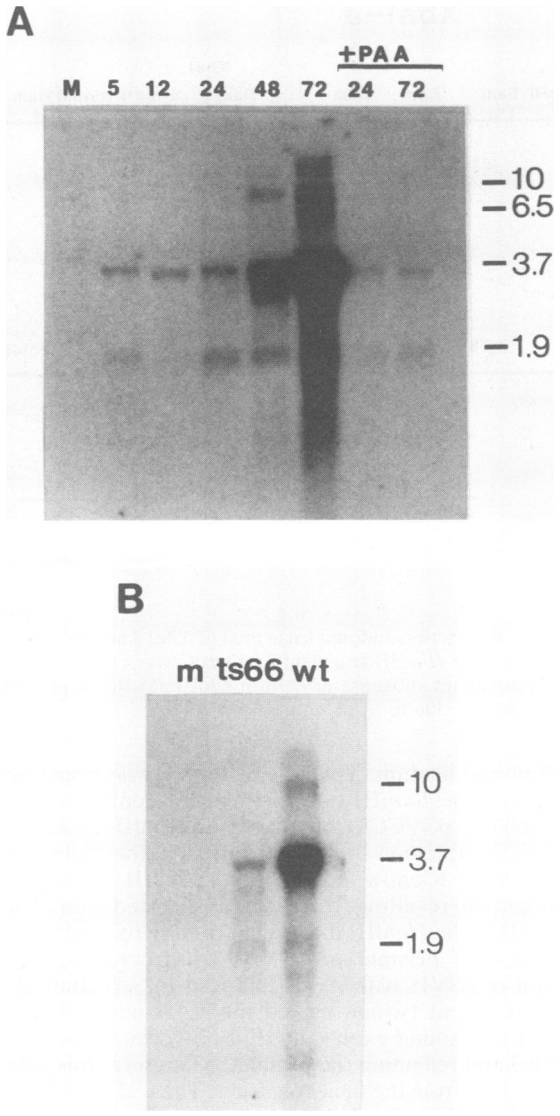

FIG. 2. Analysis of RNA expression from the pp65 gene. (A) Whole-cell RNA was isolated at the indicated hours after infection from cells infected with CMV. Mock-infected cell RNA (M) (1.0 μg) and RNA from an equivalent number of infected cells were analyzed. The time course blot was hybridized with a single-stranded RNA probe transcribed from pGHH1500 (1.5-kb *Hin*dIII fragment of *Xba*I C cloned into pGEM-3Z). (B) Cytoplasmic RNA was isolated at 72 h after infection at the nonpermissive temperature (39.5°C) from cells infected with *ts*66 or WT CMV (wt) or from mock-infected cells (m). RNA from an equivalent number of cells was analyzed. The blot was hybridized with the 1.5-kb *Hin*dIII fragment of *Xba*I C. Sizes of RNAs (in kilobases) are indicated to the right of the blots. The 4-kb pp65 RNA is consistently detected as 3.7 kb in our gel system.

Activation of the pp65 promoter in transient studies with superinfecting virus. Since pp65 RNA expression occurs maximally at late times with low levels of early expression, we investigated transient expression of the pp65 promoter and its activation by superinfecting virus. To ensure that activation of the pp65 promoter was orientation specific, a control experiment was performed to investigate activation of the promoter when cloned in the opposite orientation. p65EHCAT, pP65CAT, and pP65CATrev were transfected into HF cells and superinfected as described in Materials and Methods. Cells were harvested 72 h after infection, cell extracts were prepared, and activation of the promoters was assessed. Activation of pP65CAT and p65EHCAT occurred at approximately equal levels, whereas pP65CATrev was not activated (Table 1). Therefore, the pp65 promoter constructs require viral gene products for activation and the promoter functions only in its proper orientation.

Effect of IE gene products on promoter activation. Since

TABLE 1. Quantitation of pp65 promoter activity

Expt no. and target	Effector	% Acetylation	CAT activity[a]
Expt 1[b]			
p65EHCAT	Mock	0	
	CMV	25.3	101.2
pP65CAT	Mock	0	
	CMV	30.4	121.6
pP65CATrev	Mock	0	
	CMV	0	
Expt 2[c]			
p65EHCAT	pSVOd	0	
	pSVH	11.6	
	pSVCC3	1.2	
	pAcc	0.8	
	pSVCC3+pAcc	7.9	

[a] CAT activity is defined as percent acetylation occurring in a standard reaction of 145 μl containing 20 μl of extract. When less extract was used to produce a linear reaction, the percent acetylation was multiplied by the dilution factor to obtain CAT activity.
[b] Experiment 1 investigated activation by superinfecting virus.
[c] Experiment 2 investigated the effect of IE proteins on promoter activation.

activation of p65EHCAT occurs after virus infection, we chose to address the effect of IE proteins on the expression of the pp65 promoter. To accomplish this, we utilized plasmid vectors capable of expressing proteins from IE region 1 (pSVCC3), IE region 2 (pAcc), or IE regions 1 and 2 (pSVH). p65EHCAT was cotransfected with the indicated plasmids, and 48 h later, cell extracts were analyzed for CAT. pSVOd, which was used in constructing the IE plasmids, was incapable of activating the pp65 promoter (Table 1). When transfected individually, IE region 1 or 2 failed to activate the pp65 promoter to significant levels. However, IE 1 and 2, when cotransfected (pAcc and pSVCC3), activated p65EHCAT to approximately the same level as pSVH. The relative increase in promoter activation by pSVH was 7- to 10-fold when compared with either IE 1 or 2 (Table 1). Therefore, IE regions 1 and 2 are both required and function together for activation of the pp65 promoter.

Time course analysis of pp65 promoter activation. Since pp65 RNA is expressed early and amplified late and because the pp65 promoter-CAT construct was activated by superinfecting virus, we chose to address activation of p65EHCAT during CMV replication. To determine the relative time of activation of the pp65 promoter, HF cells were transfected with p65EHCAT, superinfected with CMV, and harvested at the indicated times. In addition, transfected cells were infected with CMV in the presence or absence of PAA. The data demonstrates that transfection followed by mock infection resulted in no activation of the promoter (Table 2). By 12 h after infection, the promoter was activated to low levels, which continued to increase through 48 h. Also, significant levels of activation occurred in the presence of PAA, although activity was reduced to levels seen at early times (24 h). Therefore, promoter activation during CMV infection indicates that the pp65 promoter is activated early and reaches a maximum activation by 48 h after infection.

Analysis of promoter deletion mutants. To determine the minimal promoter sequence necessary for virus activation, a set of promoter deletion mutants was constructed (Fig. 3). These mutants were tested for activation by superinfection with CMV or by cotransfection with pSVH as described above. Deletion to −81 (p65dl3CAT) resulted in little change

TABLE 2. Quantitation of p65EHCAT time course activation

Treatment and time (h)[a]	% Acetylation	CAT activity[b]
M[c] (72)	0	
5	0	
12	1.7	6.8
24	11.4	45.6
48	37.1	148.4
72	36.0	144.0
M + PAA (72)	0	
24 + PAA	13.7	54.8
72 + PAA	9.6	38.4

[a] Cells were harvested at the indicated times after infection.
[b] CAT activity is as defined in footnote *a* of Table 1.
[c] M, Mock infected.

in activation when compared with p65EHCAT (Table 3). Similarly, p65dl12CAT (−65) and p65dl13CAT (−61) were activated to approximately the same level as the WT promoter. However, activation of p65dl23CAT (−52) and p65dl4CAT (−22), the latter being deleted through the TATA box, were reduced significantly when compared with WT. This dramatic loss of activity occurred after the deletion of only 9 nucleotides. Therefore, sequences 3′ of −61 are important for expression of this late gene, with sequences from −61 to −52 being critical. Sequencing of the pp65 promoter and the promoter deletion mutants revealed an 8-nucleotide sequence, ATTTCGGG, at −58 to −51 that was deleted during construction of p65dl23CAT (Fig. 4). This sequence is repeated at +93 and has an inverted repeat at +67. This data suggests that this octamer sequence may be critical for activation by viral IE proteins. However, IE gene products did not completely activate the pp65 promoter to the same level as superinfecting virus (Table 3). Although IE proteins are necessary to activate the pp65 promoter, other viral gene products may contribute to complete activation.

DISCUSSION

To better understand late gene regulation in CMV-infected cells, we analyzed expression of the pp65 gene, which is abundantly transcribed at late times. Others have investigated expression of pp65 (10) and have concluded that maximal transcription of pp65 RNA occurred at early times. Our results conflict with their data but could be explained by the fact that our analyses were performed using RNA from an equivalent number of infected cells, rather than an equivalent quantity of RNA. Here we demonstrated that pp65 RNA was not transcribed abundantly until late times and that low levels of expression occurred at 24 h in the presence or absence of PAA. In addition, our studies with *ts*66 demonstrated that low levels of pp65 RNA expression occurred at the nonpermissive temperature and that these levels were relatively equal to pp65 RNA present in WT-infected cells treated with PAA. Therefore, in contrast to other late genes which absolutely require DNA replication, pp65 mRNA expression was detected in the absence of DNA replication. By these criteria, we conclude that the pp65 gene is an early-late gene requiring DNA replication for maximal expression.

To address potential mechanisms involved in pp65 gene regulation, we constructed the promoter into an indicator plasmid containing the gene for CAT. When removed from the viral genome, the pp65 promoter was activated to

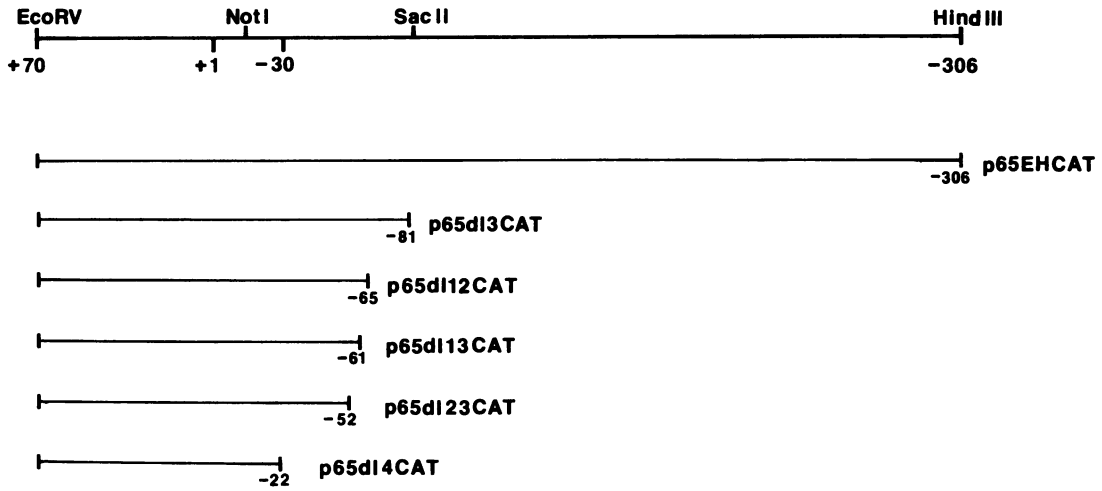

FIG. 3. Map of the pp65 promoter and location of the promoter deletion mutants. p65dl3CAT was constructed from p65EHCAT by deletion of sequences −306 (*Hin*dIII) to −84 (*Sac*II). All other deletion mutants were constructed as described in the text. Numbers given are in reference to the distance from the CAP site (+1).

significant levels at early times. This is in contrast to RNA studies in which low levels of expression occurred at early times. Clearly, pp65 RNA levels increased dramatically only late in infection and after viral DNA replication. However, this dramatic change was not reflected in the activation of the promoter-CAT construct. Therefore, additional regulatory events occurring within the viral genome may confer more stringent control of promoter activation than that which occurs in our transient assay system.

However, expression of the pp65 gene is due, at least in part, to regulation by sequences contained within the promoter. The 8-nucleotide sequence, ATTTCGGG (at −51), plays a role in the activation of the pp65 promoter by IE gene products, since deletion into the sequence prevents promoter activation. The 8-nucleotide sequence is repeated at +93, and an inverted repeat (CCCGAAAT) is located at +67. The repeat at +93 is not contained within our promoter-CAT construct, and the function of these additional repeats are unclear but are currently under investigation.

IE 1 and 2 proteins are necessary for activation of the pp65 promoter and may be part of the transcription complex. This is consistent with our studies using the CMV DNA polymerase promoter in which both IE 1 and 2 were necessary for activation of this early gene (Stenberg et al., unpublished data). While Hermiston et al. (15) and Pizzorno et al. (28) have demonstrated that IE 2 proteins are sufficient to activate heterologous promoters, we consistently found that IE 1 and 2 were required for activation of CMV promoters. However, other viral proteins binding to this domain cannot be ruled out since IE 1 and 2 could only partially activate the promoter relative to activation by superinfecting virus. Activation by CMV was consistently 10-fold higher than activation by IE 1 and 2. While superinfection is notably more efficient than cotransfection, we do not see this difference when the DNA polymerase promoter is analyzed in a similar manner (A. S. Depto and R. M. Stenberg, unpublished data). Therefore other viral proteins may work with IE 1 and 2 proteins to enhance their effect. The interaction of viral and

TABLE 3. Activity of pp65 promoter deletion mutants

Target	Activation by CMV			Activation by IE gene products		
	Effector	% Acetylation	CAT activity[a]	Effector	% Acetylation	CAT activity
p65EHCAT	M	0		pSVOd	0	
	CMV	37.3	149.2	pSVH	17.5	17.5
p65dl3CAT	M	0		pSVOd	0	
	CMV	31.9	127.6	pSVH	10.8	10.8
p65dl12CAT	M	0		pSVOd	0	
	CMV	28.7	114.8	pSVH	14.2	14.2
p65dl13CAT	M	0		pSVOd	0	
	CMV	32.8	131.2	pSVH	16.1	16.1
p65dl23CAT	M	0		pSVOd	0	
	CMV	6.7	26.8	pSVH	2.7	2.7
p65dl4CAT	M	0		pSVOd	0	
	CMV	3.4	13.6	pSVH	0.9	0.9

[a] CAT activity is as defined in footnote *a* of Table 1.

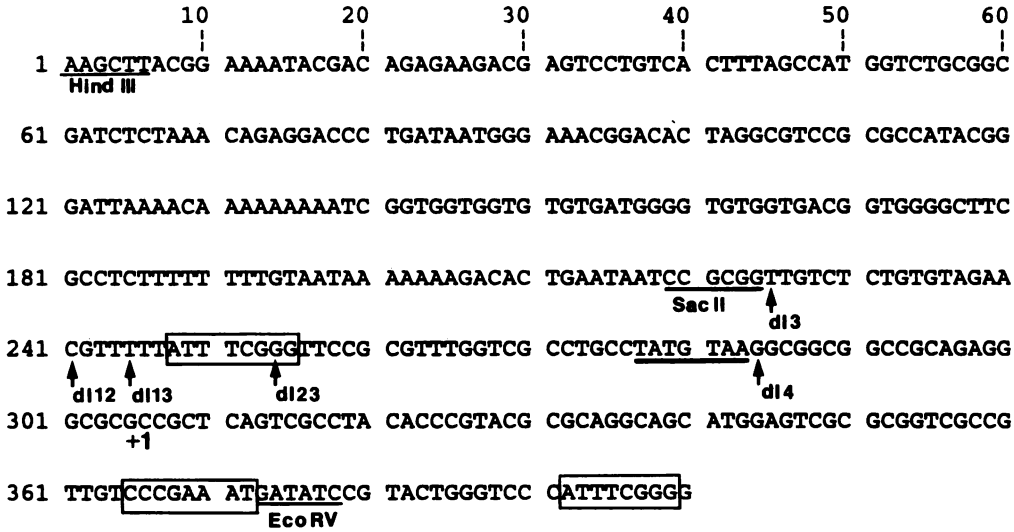

FIG. 4. Sequence of the pp65 promoter region. *Hin*dIII and *Eco*RV sites are indicated. Abbreviations for deletion mutants are as indicated: dl3 (p65dl3CAT), dl12 (p65dl12CAT), dl13 (p65dl13CAT), dl23 (p65dl23CAT), and dl4 (p65dl4CAT). The *Sac*II site used to generate p65dl3CAT is also indicated. The repeat ATTTCGGG and inverted repeat CCCGAAAT are boxed, and the TATA homology is underlined. The last upstream nucleotide of each promoter deletion construct is indicated by an arrow. +1 indicates the CAP site. Sequence data shown is the AD169 sequence from Ruger et al. (30). The Towne pp65 promoter has been sequenced from nucleotides 200 to 376 and is homologous to AD169, except for a T-to-C change at nucleotide 264.

cellular proteins with the pp65 promoter is currently being addressed.

It is unclear if the sequences involved in activation of the pp65 promoter are important for activation of other CMV promoters. Computer analysis of a number of other CMV early and late promoters revealed no homology to the ATTTCGGG sequence, although other repeats and inverted repeats, including octameric sequences, could be found in those promoters (4, 5, 13, 14, 16–18, 33). Examination of promoter regulatory sequences from other viral or cellular genes also did not reveal any similarity to the pp65 octameric sequence (1, 2, 8, 20, 27, 29, 32). However, it should be noted that mutational analysis and DNA-binding studies have shown that a number of these regulatory sequences contain only a few nucleotides important in protein binding and subsequent promoter activation (1, 29).

Further analysis of different CMV early and late genes will be necessary to understand the exact mechanisms responsible for regulation. Studies need to be performed to further characterize the interaction of *cis*-acting promoter sequences with viral and host cell DNA-binding proteins at early and late times. Such analyses will permit a more thorough understanding of the mechanisms involved in CMV gene regulation.

ACKNOWLEDGMENTS

We express our sincere thanks to James Fortney for excellent technical assistance and to Jeannine Strobl, David Yelton, and Stephen Young for helpful comments. We also thank Mark Stinski for providing *Xba*I clones and Jeannine Strobl for providing pSVOCATΔintron.

This study was supported by Public Health Service grant AI 23313 from the National Institutes of Health.

LITERATURE CITED

1. **Baldwin, A. S., Jr., and P. A. Sharp.** 1987. Binding of a nuclear factor to a regulatory sequence in the promoter of the mouse *H-2K*^{b} class I major histocompatibility gene. Mol. Cell. Biol. 7:305–311.
2. **Bohmann, D., W. Keller, T. Dale, H. R. Scholer, G. Tebb, and I. W. Mattaj.** 1987. A transcription factor which binds to the enhancers of SV40, immunoglobulin heavy chain gene, and U2 snRNA genes. Nature (London) **325**:268–272.
3. **Campos, R., and L. P. Villarreal.** 1982. An SV40 deletion mutant accumulates late transcripts in the paranuclear extract. Virology **119**:1–11.
4. **Cranage, M. P., G. L. Smith, S. E. Bell, H. Hart, C. Brown, A. T. Bankier, P. Tomlinson, B. G. Barrell, and T. C. Minson.** 1988. Identification and expression of a human cytomegalovirus glycoprotein with homology to the Epstein-Barr virus BXLF2 product, varicella-zoster virus gpIII, and herpes simplex virus type 1 glycoprotein H. J. Virol. **62**:1416–1422.
5. **Davis, M. G., and E. S. Huang.** 1985. Nucleotide sequence of a human cytomegalovirus DNA fragment encoding a 67-kilodalton phosphorylated viral protein. J. Virol. **56**:7–11.
6. **DeMarchi, J. M.** 1981. Human cytomegalovirus DNA: restriction enzyme cleavage maps and map locations for immediate early, early, and late RNAs. Virology **114**:23–38.
7. **Demarchi, J. M., G. A. Schmidt, and A. S. Kaplan.** 1980. Patterns of transcription of human cytomegalovirus in permissively infected cells. J. Virol. **35**:227–286.
8. **Falkner, F., R. Mocikat, and H. G. Zachau.** 1986. Sequences closely related to an immunoglobulin gene promoter/enhancer element occur also upstream of other eukaryotic and of prokaryotic genes. Nucleic Acids Res. **14**:8819–8827.
9. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.
10. **Geballe, A. P., F. S. Leach, and E. S. Mocarski.** 1986. Regulation of cytomegalovirus late-gene expression: γ genes are controlled by posttranscriptional events. J. Virol. **57**:864–874.
11. **Goins, W. F., and M. F. Stinski.** 1986. Expression of a human cytomegalovirus late gene is posttranscriptionally regulated by a 3′-end processing event occurring exclusively late after infection. Mol. Cell. Biol. **6**:4202–4213.
12. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143–190. *In* D. M. Glover (ed.), DNA cloning: a practical approach. vol. 2. IRL Press, Oxford.
13. **Greenaway, P. J., and G. W. G. Wilkinson.** 1987. Nucleotide sequence of the most abundantly transcribed early gene of

human cytomegalovirus strain AD169. Virus Res. 7:17–31.
14. **Heilbronn, R., G. Jahn, A. Burkle, U. Freese, B. Fleckenstein, and H. zur Hausen.** 1987. Genomic localization, sequence analysis, and transcription of the putative human cytomegalovirus DNA polymerase gene. J. Virol. **61**:119–124.
15. **Hermiston, T., C. Malone, P. Witte, and M. Stinski.** 1987. Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter. J. Virol. **61**:3214–3221.
16. **Jahn, G., T. Kouzarides, M. Mach, B. C. Scholl, B. Plachter, B. Traupe, E. Preddie, S. C. Satchwell, B. Fleckenstein, and B. G. Barrell.** 1987. Map position and nucleotide sequence of the gene for the large structural phosphoprotein of human cytomegalovirus. J. Virol. **61**:1358–1367.
17. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, K. Weston, P. Tomlinson, and G. G. Barrell.** 1987. Large-scale rearrangement of homologous regions in the genomes of HCMV and EBV. Virology **157**:397–413.
18. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, K. Weston, P. Tomlinson, and B. G. Barrell.** 1987. Sequence and transcription analysis of the human cytomegalovirus DNA polymerase gene. J. Virol. **61**:125–133.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Mantovani, R., N. Malgaretti, B. Giglioni, P. Comi, N. Cappellini, S. Nicolis, and S. Ottolenghi.** 1987. A protein factor binding to an octamer motif in the γ-globulin promoter disappears upon induction of differentiation and hemoglobin synthesis in K562 cells. Nucleic Acids Res. **15**:9349–9364.
21. **McDonough, S. H., and D. H. Spector.** 1983. Transcription in human fibroblasts permissively infected by human cytomegalovirus strain AD169. Virology **125**:31–46.
22. **McDonough, S. H., S. I. Staprans, and D. H. Spector.** 1985. Analysis of the major transcripts encoded by the long repeat of human cytomegalovirus strain AD169. J. Virol. **53**:711–718.
23. **Mellon, P., V. Parker, Y. Gluzman, and T. Maniatis.** 1981. Identification of DNA sequences required for transcription of the human α1 globin gene in a new SV40-host-vector system. Cell **27**:279–288.
24. **Meyer, H., A. T. Bankier, M. P. Landini, C. M. Brown, B. G. Barrell, B. Ruger, and M. Mach.** 1988. Identification and procaryotic expression of the gene coding for the highly immunogenic 28-kilodalton structural phosphoprotein (pp28) of human cytomegalovirus. J. Virol. **62**:2243–2250.
25. **Nowak, B., A. Gmeiner, P. Sarnow, A. Levine, and B. Fleckenstein.** 1984. Physical mapping of human cytomegalovirus genes: identification of DNA sequences coding for a virion phosphoprotein of 71 kDa and for a viral 65 kDa polypeptide. Virology **134**:91–102.
26. **Pande, H., S. W. Baak, A. D. Riggs, B. R. Clark, J. Shively, and J. A. Zaia.** 1984. Cloning and physical mapping of a gene fragment coding for a 64-kilodalton major late antigen of human cytomegalovirus. Proc. Natl. Acad. Sci. USA **81**:4965–4969.
27. **Perez-Mutal, J., M. Macchi, and B. Wasylyk.** 1988. Mutational analysis of the contribution of sequence motifs within the IgH enhancer to tissue specific transcriptional activation. Nucleic Acids Res. **16**:6085–6096.
28. **Pizzorno, M. C., P. O'Hare, L. Sha, R. L. LaFamina, and G. S. Hayward.** 1988. *trans*-Activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. J. Virol. **62**:1167–1179.
29. **Raychaudhuri, P., R. Rooney, and J. R. Nevins.** 1987. Identification of an E1A-inducible cellular factor that interacts with regulatory sequences within the adenovirus E4 promoter. EMBO J. **6**:4073–4081.
30. **Ruger, B., S. Klages, B. Walla, J. Albrecht, B. Fleckenstein, P. Tomlinson, and B. Barrell.** 1987. Primary structure and transcription of the genes coding for the two virion phosphoproteins pp65 and pp71 of human cytomegalovirus. J. Virol. **61**:446–453.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **SivaRaman, L., and B. Thimmappaya.** 1987. Two promoter specific host factors interact with adjacent sequences in an E1A-inducible adenovirus promoter. Proc. Natl. Acad. Sci. USA **84**:6112–6116.
33. **Staprans, S. I., and D. H. Spector.** 1986. 2.2-Kilobase class of early transcripts encoded by cell-related sequences in human cytomegalovirus strain AD169. J. Virol. **57**:591–602.
34. **Stenberg, R. M., and L. I. Pizer.** 1982. Herpes simplex virus-induced changes in cellular and adenovirus RNA metabolism in an adenovirus type 5-transformed human cell line. J. Virol. **42**:474–487.
35. **Stenberg, R. M., and M. F. Stinski.** 1985. Autoregulation of the human cytomegalovirus major immediate-early gene. J. Virol. **56**:676–682.
36. **Stenberg, R. M., D. R. Thomsen, and M. F. Stinski.** 1984. Structural analysis of the major immediate early gene of human cytomegalovirus. J. Virol. **49**:190–199.
37. **Stenberg, R. M., P. R. Witte, and M. F. Stinski.** 1985. Multiple spliced and unspliced transcripts from human cytomegalovirus immediate-early region 2 and evidence for a common initiation site within immediate-early region 1. J. Virol. **56**:665–675.
38. **Stinski, M. F.** 1978. Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. J. Virol. **26**:686–701.
39. **Stinski, M. F., D. R. Thomsen, R. M. Stenberg, and L. C. Goldstein.** 1983. Organization and expression of the immediate early genes of human cytomegalovirus. J. Virol. **46**:1–14.
40. **Strohman, R., P. Moss, J. Micou-Eastwood, D. Spector, A. Przybyla, and B. Peterson.** 1977. Messenger RNA for myosin polypeptides: isolation from single myogenic cell cultures. Cell **10**:265–273.
41. **Thomsen, D. R., and M. F. Stinski.** 1981. Cloning of the human cytomegalovirus genome as endonuclease *XbaI* fragments. Gene **16**:207–216.
42. **Wathen, M. W., and M. F. Stinski.** 1982. Temporal patterns of human cytomegalovirus transcription: mapping the viral RNAs synthesized at immediate early, early, and late times after infection. J. Virol. **41**:462–477.
43. **Wathen, M. W., D. R. Thomsen, and M. F. Stinski.** 1981. Temporal regulation of human cytomegalovirus transcription at immediate early and early times after infection. J. Virol. **38**: 446–459.