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The Role of ATF in Regulating the Human Cytomegalovirus DNA Polymerase (UL54) Promoter during Viral Infection

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Previous analysis of the human cytomegalovirus (HCMV) DNA polymerase (UL54) early gene promoter demonstrated that transcriptional activation of this gene is dependent upon the interaction of cellular transcription factors with viral transactivators (J. A. Kerry, M. A. Priddy, T. Y. Jervey, C. P. Kohler, T. L. Staley, C. D. Vanson, T. R. Jones, A. C. Iskenderian, D. G. Anders, and R. M. Stenberg, J. Virol. 70:373–382, 1996). A sequence element, IR1, was shown to be the primary regulatory element of this promoter in transient assays. However, assessment of this element in the context of the viral genome revealed IR1-independent activation at late times after infection. To extend these studies, we aim to identify additional sequence elements involved in the activation of the UL54 promoter. Our present studies demonstrate that the level of binding of proteins to the ATF site in the UL54 promoter is enhanced by viral infection. Furthermore this increase is sensitive to treatment with phosphonoacetic acid (PAA), a DNA synthesis inhibitor. These data suggest that the increase in the level of ATF binding activity is regulated, either directly or indirectly, by HCMV late gene expression. By using specific antibodies, we determined that ATF-1 was a major component of the proteins binding to the UL54 ATF site at late times. In addition, we have demonstrated direct binding of recombinant ATF-1 to the UL54 ATF site. To assess the biological significance of these events, a recombinant virus construct was generated that contained the UL54 promoter with a mutation in the ATF site regulating expression of the chloramphenicol acetyltransferase (CAT) reporter gene inserted between open reading frames US9 and US10. Analysis of this virus (RVATFmCAT) revealed that mutation of the ATF site does not alter the kinetics of UL54 promoter activation. However, levels of CAT mRNA and activity were reduced by 5- to 10-fold compared to those of the wild-type promoter at all stages of infection. These findings indicate that ATF-1 can regulate the levels of UL54 promoter activity at both early and late times. Furthermore, these results imply that HCMV can regulate the activity of cellular factors involved in early gene regulation.

Human cytomegalovirus (HCMV) is a major cause of complications in AIDS and organ transplant patients (6). Primary infection or reactivation of latent HCMV in immunocompromised patients can result in severe symptoms such as retinitis, pneumonia, and encephalitis. The factors which regulate the processes of replication and reactivation of HCMV are not clearly understood. However, cellular factors are thought to play a crucial role in these events. Activation of HCMV immediate-early (IE) gene expression requires cellular transcription factors (51). In addition, cellular transcription factors and viral transactivators are essential for early gene activation (9, 17, 21, 26, 33, 36, 49, 55, 57).

Activation of the major IE promoter requires cellular factors such as NF-κB, ATF/CREB, and NF-1 (8, 14, 16, 18, 45). Virus binding to the cellular receptor results in the up-regulation of some factors required for IE gene transcription (3, 4). In addition, monocyte differentiation can up-regulate cellular factors required for IE gene expression (54). In some nonpermissive cell lines, IE gene expression is restricted, indicating that IE proteins are crucial for productive infection (48, 54, 61). However, in other instances, IE gene expression occurs but the virus does not progress in the replicative cycle (44, 46, 61). These studies suggest that cellular factors required for early gene activation may also be important in regulating HCMV replication. To date, early gene promoters have been shown to be activated by a diverse group of cellular factors, including E2F, AP-1, and USF (7, 17, 25, 28, 29, 50, 52, 53, 59). Recently, it has been shown that CREB-A can interact with the IE protein IE86 and is involved in the activation of the HCMV early UL112-113 promoter (33). Many HCMV early gene promoters contain potential binding sites for ATF/CREB proteins (1, 7, 25, 28, 33, 50, 52, 53, 62). Thus, ATF/CREB may be involved in the regulation of multiple HCMV early genes.

The ATF/CREB group of transcription factors consists of a family of highly regulated, related proteins (35). These factors are members of a broader class of transcription factors known as basic-leucine zipper proteins (40, 42). Other members of this group include c-*jun* and c-*fos* (42). Dimerization partners are specific for particular leucine zipper proteins and can affect DNA binding specificity as well as binding stability (15, 19, 31, 38). Thus, dimerization among ATF/CREB subtypes is likely to have a significant impact on transcriptional activation by these proteins. The activity of ATF/CREB proteins is also regulated at the levels of transcription, splicing, and posttranslational modification (35, 38, 43). Viral proteins such as E1a and Tax can also alter the DNA binding specificity of ATF/CREB subtypes (5, 37, 60). Thus, the activity of ATF/CREB proteins is highly regulated at multiple levels. This level of regulation likely reflects the importance of these factors in the cell. Cellular genes that can be activated by ATF/CREB include those involved in gluconeogenesis, growth hormone factor 1, DNA polymerase β, the tumor suppresser gene RB, cyclin A, and the proto-oncogene c-*fos* (11, 35, 41, 63).

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In order to better characterize early gene regulation in HCMV-infected cells, we have been analyzing the regulation of the HCMV DNA polymerase promoter (UL54) as a prototypical early gene (25, 26, 55). Our earlier studies identified the primary regulatory element, IR1, critical for UL54 promoter activation at early times after infection (25, 26). However, at late times after infection, IR1-independent activation of the UL54 promoter occurs (26). Our present studies aim to characterize additional sequence elements required for UL54 promoter activation. These analyses have focused on the role of ATF in regulating the UL54 promoter. Previous studies demonstrated that mutation of this site resulted in a slight but consistent decrease in UL54 promoter activity in transient assays (25). Our studies herein demonstrate that HCMV infection up-regulates the level of proteins capable of binding to the UL54 ATF promoter element at late times after infection. The protein binding to the UL54 promoter element was identified by gel supershift analysis as ATF-1. Assessment in the context of the viral genome revealed that mutation of the ATF site in the UL54 promoter reduces promoter activity at both early and late times after infection. Together, these studies show that ATF contributes to the activation of the UL54 promoter.

MATERIALS AND METHODS

Gel mobility shift analysis. Nuclear extracts were prepared exactly as previously described (25). Briefly, human fibroblasts were infected with HCMV Towne at 5 to 10 PFU per cell at confluence. Extracts were prepared, at the indicated times after infection, by a modification of the Dignam procedure (12, 25). Nuclear extracts (10 μg) were incubated with 2 μg of poly(dI)-poly(dC) and competitor DNAs for 10 min at room temperature in 50% buffer D (12). Radiolabeled probe (30,000 to 40,000 cpm, 0.5 to 1 ng of DNA) was then added, and the incubation continued for 20 min. For supershift analysis, 1 μg of the appropriate antibody was added at this time, and the incubation was allowed to proceed for a further 20 min. Samples were subjected to polyacrylamide gel electrophoresis in 1/2× Tris-borate-EDTA (TBE). Gels were then dried and subjected to autoradiography.

Probe DNA (DR1ATF) consisted of a 40-bp fragment of the UL54 promoter containing a direct repeat element (DR1) as well as the ATF site (25, 55). Gel shift assays were performed with the wild-type DR1ATF fragment labeled at the 5′ ends with [γ-^{32}P]ATP and T4 polynucleotide kinase. Mutations of the DR1 and ATF sites were cloned as oligonucleotide fragments into the *Hin*dIII site of the pd25/26CAT plasmid (10, 25, 55). The oligonucleotides contained base substitutions that mutated either the DR1 element, the ATF site, or both (25). Competitor fragments were then isolated by *Hin*dIII digestion of the resulting plasmids followed by polyacrylamide gel electrophoresis. The oligonucleotides used to generate these mutants are indicated below with the mutated nucleotides underlined:

DRmATF, 5′CCGAAGCTTCGGGCAACACGTGACGTCGGGCAGAAGCTTGCC
DR1ATFm, 5′CCGAAGCTTCGGGACCACCGTGAATTCGGGCAGAAGCTTGCC
DRmATFm, 5′CCGAAGCTTCGGGCAACACGTGAATTCGGGCAGAAGCTTGCC
primer, 5′GGCAAGCTTCTGCCC

Recombinant ATF-1 protein (rATF-1) utilized in these experiments was obtained from Santa Cruz Biotechnology (Santa Cruz, Calif.). Antibodies were also obtained from Santa Cruz Biotechnology and include an ATF-1 monoclonal antibody (25C10G) that cross-reacts with CREB-1 and CREM-1; ATF-2-specific monoclonal antibody (F2BR-1); CREB-1-specific rabbit polyclonal antibody (C-21); c-Jun/AP-1(D) goat polyclonal antibody that cross-reacts with c-Jun, JunB, and JunD; and a Fos monoclonal antibody (4-10G) that cross-reacts with c-Fos, FosB, Fra-1, and Fra-2.

Generation and isolation of recombinant virus. The virus RVpolCAT, containing the UL54 promoter regulating the expression of the chloramphenicol acetyltransferase (CAT) reporter gene between the US9 and US10 genes, has been previously described (30). The virus RVATFmCAT was generated by our modified calcium phosphate transfection protocol (22, 23, 30). Briefly, the *Nco*I to *Sal*I fragment of pPolATFmCAT (25) containing a 2-nucleotide mutation in the ATF site of the UL54 promoter was cloned into *Nco*I-*Sal*I-digested pRC2 (26). This construct was linearized at the unique *Xmn*I restriction site and was subsequently cotransfected with RV134 DNA (22, 23). Purified recombinant viral isolates were then selected from the transfectant population exactly as described in reference 30.

Growth curve analysis of the recombinant virus. Growth curve analysis of the recombinant viruses was performed as described (23). Briefly, human fibroblast cells were infected with the appropriate virus at a multiplicity of infection (MOI) of 2. Cells and supernatant were harvested daily, and virus titers were assessed by standard techniques.

Southern blot analysis. Human fibroblast (HF) cells infected with recombinant viruses were harvested for total-cell DNA at 100% CPE (26, 27, 30). The infected-cell DNAs were digested with *Eco*RI and *Xho*I and subjected to electrophoresis on 1.2% agarose gels in 1× TBE buffer. The gels were transferred to positively charged nylon membranes (Biodyne B; Gibco Life Technologies, Grand Island, N.Y.) and hybridized to a probe for chloramphenicol acetyltransferase (CAT) or the *Hin*dIII X fragment of AD169, using the Genius detection system (Boehringer-Mannheim Corporation, Indianapolis, Ind.).

Northern blot analysis. Total-cell RNA was isolated from infected cells, using the RNeasy system (Qiagen, Chatsworth, Calif.). Equal quantities of RNA (5 μg) were subjected to Northern blot analysis and hybridized to ^{32}P-radiolabeled probe for CAT. RNA levels were quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, Calif.). MOI was assessed by stripping the Northern blots and reprobing with a ^{32}P-radiolabeled probe to the endogenous pp28 gene (UL99). These results were confirmed by assessing the levels of the UL98 transcript which is 3′ coterminal with the UL99 transcript (25, 26). The level of US9 gene expression was assessed in the same manner, using a probe specific for the US9-US8 open reading frames.

RESULTS

HCMV regulates proteins binding to the UL54 ATF site. Our previous analysis of the UL54 promoter indicated that a sequence element, IR1, located from −53 to −45 was the primary element required for promoter activation in transient assays (25, 26). When assessed in the context of the viral genome, the IR1 element was determined to be critical for activation of the UL54 promoter at early times. However, IR1-independent activation of the UL54 promoter was observed at late times after infection (26). In the present study, we aim to identify additional promoter elements required for UL54 promoter activation. Our previous analysis of the UL54 promoter utilizing transient assays revealed that mutation of the ATF element located from −88 to −80 resulted in a slight but consistent drop in promoter activity (25). In addition, cloning of UL54 promoter sequences, including the ATF element, upstream of a minimal promoter conferred activation by viral IE proteins (55). To assess the potential for the ATF site to regulate the UL54 promoter in infected cells, the ability of nuclear proteins to bind to a DNA probe containing the ATF site of the UL54 promoter (DR1ATF probe) was examined (Fig. 1A). This analysis revealed that proteins present in both uninfected and infected cell nuclear extracts were capable of binding to the DR1ATF probe. At early times after infection, the level of ATF binding to the DR1ATF probe was relatively low. However, a dramatic increase in binding activity was observed at late times after infection. This increase in the level of DNA-binding activity was sensitive to treatment of the infected cells with phosphonoacetic acid (PAA), a DNA synthesis inhibitor. This finding suggests that ATF DNA-binding activity is up-regulated, either directly or indirectly, by HCMV late gene expression.

The DR1ATF probe consists of sequences from −99 to −75 of the UL54 promoter and also contains a copy of a direct repeat element, DR1 (25, 55). Because the probe utilized in this study contains two potential regulatory elements, the specificity of binding was assessed by competition analysis (Fig. 1B). DNA fragments containing mutations in either the DR1 or ATF elements or both were utilized as competitors with the wild-type probe in a gel shift assay. This study revealed that DNA binding could only be competed by probes containing a wild-type ATF element (DR1ATF and DRmATF). The two DNA fragments containing a 2-nucleotide mutation of the ATF site (DR1ATFm and DRmATFm) were unable to compete for binding to the wild-type probe DNA. These data strongly suggest that binding to the DR1ATF probe is specific for the ATF site.

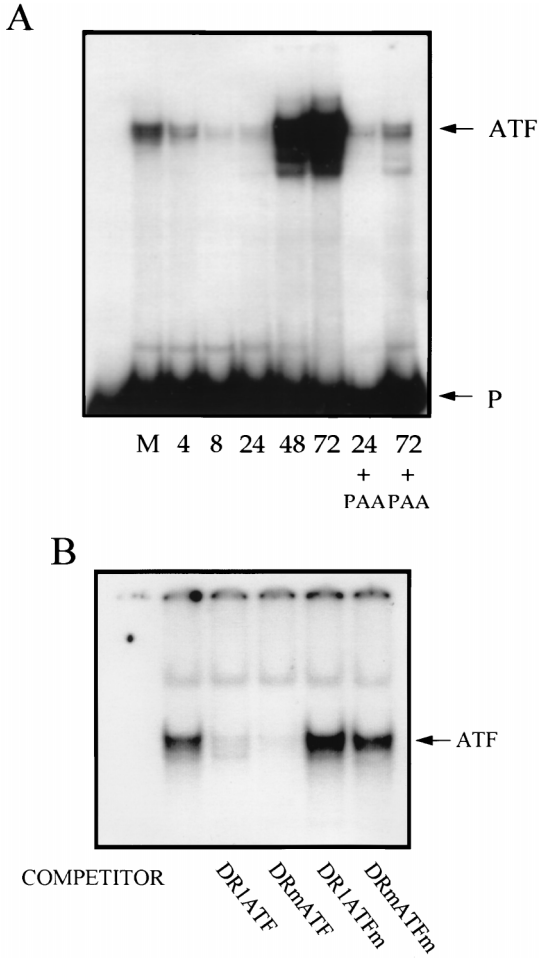

FIG. 1. (A) Time course analysis of protein binding activity. A 40-bp radiolabeled probe containing the UL54 ATF site (DR1ATF probe) was incubated with nuclear extracts prepared at the indicated times after infection. Infection was carried out in the presence or absence of PAA (200 μg/ml). Protein-DNA complexes were then analyzed by electrophoresis, using a 4% acrylamide gel in 1/2 × TBE. Mock-infected extracts (M) were prepared at 72 h. Free probe is indicated as P. The image was generated with a Hewlett-Packard ScanJet II cx with Hewlett-Packard HPDeskscan II software (Version 2.3.1) and labeled with Microsoft Powerpoint. (B) Specificity of binding to the UL54 ATF probe. The DR1ATF probe was incubated with nuclear extracts prepared 72 h after infection. Reaction mixtures were incubated in the presence or absence of 50-fold molar excess of unlabeled competitor DNAs. Protein-DNA complexes were then analyzed by electrophoresis, using a 4% acrylamide gel in 1/2 × TBE. DR1ATF, wild-type competitor DNA; DRmATF, competitor containing the DR1 site with a 4-nucleotide mutation; DR1ATFm, competitor containing the ATF site with a 2-nucleotide mutation; DRmATFm, competitor containing mutations in both the DR1 site and ATF sites.

Identification of proteins binding to the UL54 ATF site. To determine the subtype of ATF binding to the UL54 promoter element at late times, gel shift assays were performed with antibodies specific for particular ATF subtypes and related proteins (Fig. 2). Results show that antibody to ATF-1 is capable of supershifting the DNA-protein complex generated with the DR1ATF probe. In contrast, antibodies to ATF-2, c-*jun*, or c-*fos* had no effect on complex formation. The ATF-1 antibody utilized in this experiment was also capable of cross-reacting with CREB-1 and CREM proteins. In addition, ATF-1 is known to bind as a heterodimer with CREB-1 (15, 19). To assess if ATF-1 alone was binding to the probe DNA, a similar experiment was performed with antibody specific for

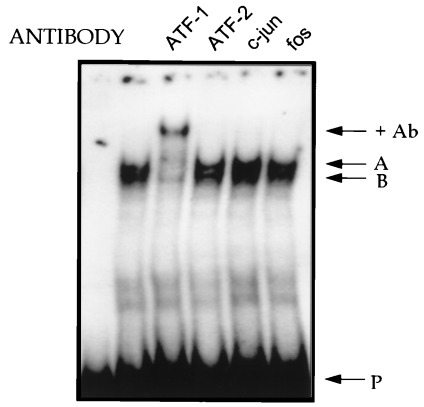

FIG. 2. Identification of the proteins binding to the UL54 ATF site. Radiolabeled DR1ATF probe was incubated with nuclear extracts prepared 72 h after infection. Reaction mixtures were incubated in the presence or absence of specific antibodies (Ab) to either ATF-1, ATF-2, c-*jun*, or c-*fos*. Protein-DNA complexes were then analyzed by electrophoresis, using a 4% acrylamide gel in 1/2 × TBE. A and B, protein-DNA complexes; P, free probe.

the CREB-1 protein (24). This analysis revealed that antibodies to CREB-1 had no effect on the complex formed with the UL54 DR1ATF probe. In contrast, the same CREB-1 antibody was capable of supershifting complexes formed with other probes containing ATF sites (24). These data imply that CREB-1 was not a component of the complex bound to the UL54 ATF site. Together, these data confirmed that ATF-1 protein present in HCMV-infected cell nuclear extracts was able to bind to the DR1ATF probe.

Analysis of the gel shift assay demonstrates the presence of two DNA-protein complexes formed with the DR1ATF probe, A and B (Fig. 2). The ATF-1 antibody was able to supershift both of these complexes. These data imply that ATF-1 is a component of both complexes A and B. To determine if ATF-1 homodimers are capable of binding directly to the UL54 ATF site, a gel shift assay was performed with rATF-1 protein (Fig. 3). This experiment shows that rATF-1 is capable of binding as a homodimer to the ATF probe. However, the mobility of the rATF-1 homodimer differs from that of both complexes A and

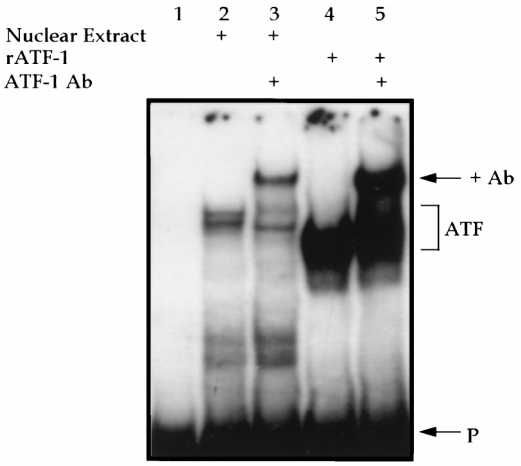

FIG. 3. rATF-1 binds directly to the UL54 ATF site. Radiolabeled DR1ATF probe was incubated with either nuclear extracts prepared at 72 h or purified rATF-1 protein. Reaction mixtures were incubated in the presence or absence of specific antibody (Ab) to ATF-1. Protein-DNA complexes were then analyzed by electrophoresis, using a 4% acrylamide gel in 1/2 × TBE. P, free probe.

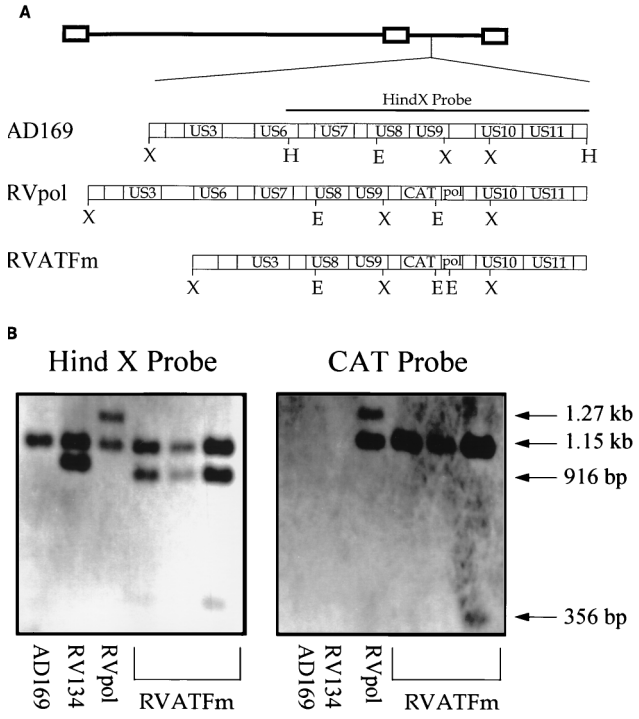

FIG. 4. (A) Map of the UL54 promoter-CAT-containing viruses. The structures of the AD169, RVpol, and RVATFm viruses are indicated. The *Hin*dX probe utilized for Southern blot analysis is shown, and relevant restriction sites are indicated. E, *Eco*RI; X, *Xho*I. (B) Southern blot analysis of recombinant viruses RVpol and RVATFm. Total cellular DNA was isolated from cells infected with the indicated virus for 72 h, digested with *Xho*I and *Eco*RI, and subjected to Southern blot analysis. The blots were hybridized with labeled probe generated from the *Hin*dIII X fragment or the isolated CAT gene, respectively. The molecular sizes of the relevant bands are indicated. RVATFm differs from the original RVpol construct by the presence of an *Eco*RI site within the ATF site of the promoter, resulting in fragments with sizes of 916 and 356 bp upon *Xho*I and *Eco*RI digestion.

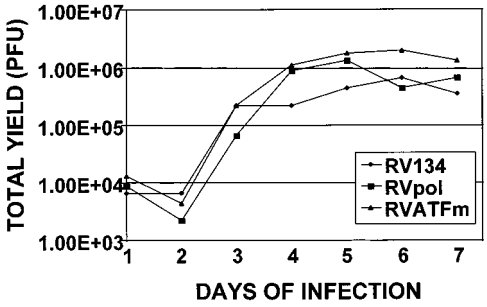

FIG. 5. Growth curve analysis of recombinant viruses. Human fibroblast cells were infected with the indicated viruses at an MOI of 2. Total virus was harvested daily, and the titer was determined by standard techniques.

B that were formed by utilization of nuclear extracts. Together, these data imply that complexes A and B may consist of ATF-1 as a heterodimer with an as yet unidentified protein.

Role of ATF in the regulation of the HCMV UL54 promoter. To assess the role of the ATF site in regulating the UL54 promoter during the course of viral infection, a UL54 promoter-CAT construct with a mutation in the ATF site was recombined into the viral genome between US9 and US10, using our previously described approach (22, 23, 26, 27, 30). Southern blot analysis of this virus (RVATFmCAT) revealed that the promoter-CAT construct was appropriately inserted into the viral genome (Fig. 4). *Eco*RI-*Xho*I digestion of the viral DNA demonstrates the presence of an additional *Eco*RI site in the promoter region as a result of the mutation in the ATF site (TGACGTC to TGAATTC). Digestion of viral DNA from the wild-type construct (RVpolCAT [30]) with *Eco*RI and *Xho*I results in two fragments with sizes of 1.27 and 1.15 kb that hybridize with the *Hin*dX probe (Fig. 4B). The presence of the additional *Eco*RI site as a result of the ATF mutation results in the conversion of the 1.27-kb fragment to two fragments with sizes of 916 and 356 bp. However, further analysis of RVATFmCAT revealed that this virus construct contains a 1.4-kb deletion in the US6-US7 gene region (Fig. 4A) (24). Because this region is nonessential for viral replication (22), the deletion should have little impact on UL54 gene regulation or viral growth. No apparent difference in the growth rates of RVATFmCAT and RVpolCAT had been observed in generating stocks of these viruses (24). However, to confirm that the 1.4-kb deletion had no effect on virus replication, a growth curve analysis of RVATFmCAT was performed. These data show that RVATFmCAT grows at a rate similar to that of RVpolCAT as well as that of the parental recombinant virus, RV134 (Fig. 5). Thus the deletion of US6-US7 has no significant impact on the ability of this virus to replicate. To confirm that the deletion in RVATFmCAT does not affect UL54 promoter activity, we assessed expression from the US9 promoter. The US9 open reading frame lies between the UL54 promoter-CAT gene and the region deleted in RVATFmCAT. This study showed that there was no significant difference in the expression of the 1.7-kb US9 transcript in RVATFmCAT-infected cells compared to that in RVpolCAT-infected cells (24). Together, these data suggest that the deletion of the US6-US7 region would likely have no impact on UL54 promoter activity.

Expression of the CAT gene in RVATFmCAT-infected cells was then assessed and compared to that of the wild-type promoter construct, RVpolCAT. Cells were infected at approximately equivalent MOIs and assessed at various times for CAT activity and CAT mRNA (Fig. 6). This analysis revealed that mutation of the ATF site in the UL54 promoter results in decreased levels of CAT activity and mRNA at all time points measured. For example, the level of CAT mRNA at 24 h postinfection was approximately 10-fold lower than that observed in RVpolCAT-infected cells. Similarly, at 72 h postinfection, mRNA levels were reduced by sixfold in RVATFmCAT-infected cells. CAT protein levels determined from CAT activity were also reduced by five- to sixfold throughout the course of infection. These results demonstrate that the ATF site in the UL54 promoter is critical for promoter activity at both early and late times after infection.

DISCUSSION

Early gene regulation is likely to be critical in determining the outcome of HCMV infection. The activation of early gene promoters requires a complex interaction between multiple viral transactivators and cellular transcription factors (9, 17, 21, 26, 33, 36, 49, 55, 57). Early genes have been shown to be regulated by a diverse group of cellular transcription factors, including CREB-A, AP-1, E2F, USF, and IR1bp (7, 17, 25, 28, 29, 33, 50, 52, 53, 59). It is difficult to anticipate how such a diverse group of factors may be regulated in different cell types in order to control HCMV replication. One hypothesis is that particular transcription factors may play a critical role in the virus replicative cycle. It is likely that these cellular transcrip-

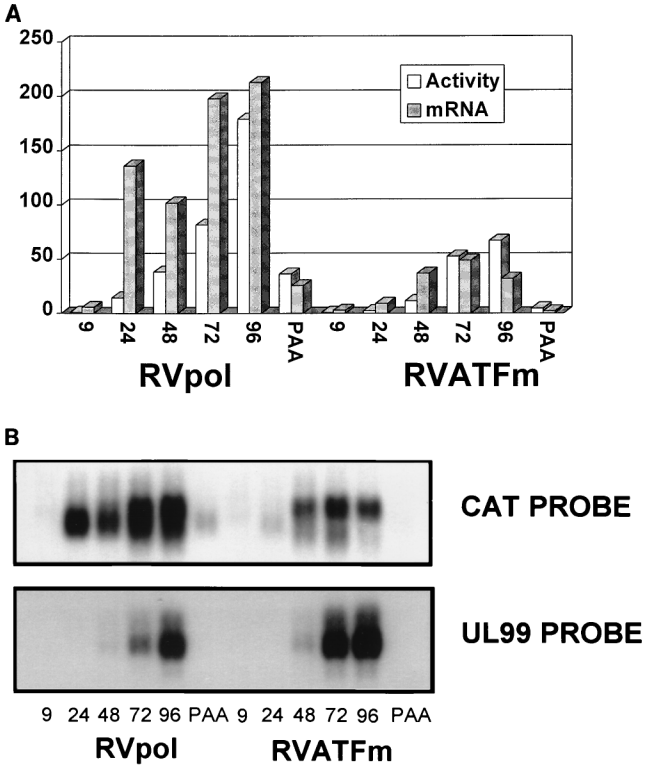

FIG. 6. Kinetics of CAT expression from recombinant viruses. HF cells were infected with 5 PFU of the indicated viruses per cell in the presence (200 μg/ml) or absence of PAA. Cells were harvested at the indicated times and assessed for CAT enzyme activity (A) or CAT RNA levels (A and B) as detected by Northern blot analysis with a ^{32}P-labeled probe specific for the CAT gene. Percent acetylation and RNA levels were quantitated by PhosphoImager analysis. Values shown in panel A were corrected for MOI by stripping the blot and reprobing it with a probe to the UL99 gene (B). Values represent typical results from two replicate experiments.

tion factors themselves are highly regulated rather than constitutively active. This hypothesis would help to explain the pattern of HCMV permissiveness in undifferentiated and differentiated macrophages (13, 20, 34, 44, 46–48, 58, 61).

It has been noted that several HCMV early promoters have ATF/CREB sites (1, 7, 25, 28, 33, 50, 53, 62). Recently, Lang et al. (33) demonstrated a role for CREB in the stimulation of the UL112-113 promoter by IE86. These studies revealed that IE86 could interact directly with CREB-A (33). Our present studies indicate that ATF-1 is involved in the regulation of the UL54 promoter. Interestingly, the DNA polymerase promoter of the closely related human herpesvirus 6 is also regulated by an ATF/CREB site (2). A major increase in the level of DNA-binding activity to the UL54 ATF site was observed at late times. This up-regulation was found to be sensitive to treatment of the infected cells with PAA, implying that HCMV late gene expression may contribute to the increase in ATF activity. An HCMV late protein, the pp71 or UL82 tegument protein, has been shown to stimulate the activity of promoters containing ATF and AP-1 sites (36). One possibility is that transcriptional regulation by pp71 is a result of the up-regulation of ATF-1 activity at the level of either transcription, heterodimerization, or phosphorylation (15, 19, 31, 38, 43). The differences in mobility of DNA-protein complexes formed with rATF-1 protein versus those present in nuclear extracts suggest that ATF-1 may bind to the UL54 ATF site as a heterodimer. The only factor known to heterodimerize with ATF-1 is CREB-1 (15, 19). ATF-1 homodimers and ATF-1/CREB-1 heterodimers are capable of binding to DNA and act as transcriptional activators (19, 31, 38). However, CREB-1 antibodies were unable to supershift the complexes formed with extracts from infected cells. ATF-1 heterodimerization with another protein has been reported, although the identity of this protein is currently unknown (32). An alternative possibility is that the difference in mobility is a result of ATF-1 phosphorylation. Phosphorylation of ATF-1 by protein kinase A can influence both the stability and mobility of rATF-1–DNA complexes (39, 43). In addition, phosphorylation of rATF-1 results in the formation of several protein-DNA complexes. This is most likely a result of phosphorylation on multiple sites within the amino-terminal region of the protein (39). Phosphorylation has been shown to enhance the transcriptional activity of ATF-1 (43). Paradoxically, this modification can also reduce the stability of ATF-1–DNA complexes (39).

Our present study demonstrates that ATF can play a critical role in the regulation of one HCMV early gene. The ATF element was found to contribute to the overall level of UL54 promoter activity at both early and late times after infection. In addition, the level of ATF binding to the UL54 ATF element was shown to be up-regulated by viral infection. These findings suggest that ATF may play an important role in HCMV gene expression and, by implication, HCMV replication. The effect of the ATF mutation on UL54 promoter activity at early times was somewhat surprising. Transient transfection analysis of this mutant indicated that mutation in the ATF site had only a slight impact on the ability of the UL54 promoter to be activated by viral IE proteins (25). In addition, the level of ATF DNA-binding activity at early times is relatively low compared to that at late times. The IE86 protein is capable of interacting with CREB-A, another member of the ATF/CREB family (33). This interaction was shown to be involved in transcriptional activation mediated via an ATF/CREB element. It is possible that a similar interaction is important for UL54 promoter activation at early times. Interestingly, the IE86–CREB-A interaction appeared to play a lesser role in regulating gene expression from an intact HCMV promoter, UL112-113, in transient assays (33). This is similar to what we observed with the ATF mutant in the UL54 promoter in transient assays (25). These findings again demonstrate the importance of assessing HCMV gene expression in the context of the viral genome. Mutation of the ATF site also resulted in a significant reduction of UL54 promoter activity in infected cells at late times. The up-regulation of ATF by HCMV infection at late times may be responsible for the late activation of the UL54 promoter. However, we cannot discount the possibility that the IE86 protein is also involved. Significant levels of IE86 are present in infected cells throughout the course of infection (56). Further experiments are required to distinguish between these possibilities.

In conclusion, these studies suggest that HCMV infection is capable of up-regulating the activity of cellular transcription factors involved in early gene regulation. Specifically, we have demonstrated that HCMV infection stimulates the level of ATF-1 DNA-binding activity. In fully permissive cells, the up-regulation of ATF at late times may be required to enhance viral replication at this stage of infection. Disruption of the ability of HCMV to regulate ATF may decrease virus replication, leading to either a latent or abortive infection. The ability of HCMV to stimulate ATF/CREB activity may therefore be crucial for determining the outcome of HCMV infection. We predicted that critical factors involved in controlling HCMV replication would be highly regulated to account for the pattern of HCMV permissiveness, latency, and reactivation. A

role for ATF in the control of viral replication is consistent with this hypothesis. We are currently determining the pathway of ATF regulation by HCMV in order to further test this hypothesis.

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