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Transcriptional control of herpesvirus gene expression: Gene functions required for positive and negative regulation

(herpes simplex virus/DNA-binding proteins/nuclear run-off transcription)

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ABSTRACT **We have used an *in vitro* nuclear run-off assay to measure the levels of transcription of specific herpes simplex virus genes at different times during a lytic infection. We analyzed the effects of inhibition of DNA replication and of defects in two herpes simplex virus regulatory proteins on the transcription of these genes. We present evidence that the transcription of the *αICP4* gene is negatively regulated during a lytic infection. The regulation of *ICP4* gene transcription requires the β protein ICP8 (where ICP = infected cell polypeptide). Transcription of the *βICP8*, *γ_1ICP5*, and γ_2 glycoprotein C (*gC*) genes was dependent on ICP4, and transcription of the *γ_2gC* gene was strongly inhibited when DNA replication was blocked. Defects in ICP8 also resulted in increased levels of transcription of the *ICP4*, *ICP8*, *ICP5*, and *gC* genes from parental viral genomes. Our results suggest that ICP8 may be important in maintaining the highly ordered cascade of viral gene expression.**

The expression of herpes simplex virus (HSV) genes is a tightly regulated process (1, 2). Upon infection of the host cell, a limited portion of the viral genome is transcribed by RNA polymerase II to produce the α (immediate early) mRNAs (3–5). Translation of α mRNAs yields the α proteins, infected cell polypeptides (ICPs) 0, 4, 22, 27, and 47. Functional α proteins are required for the accumulation of all subsequent classes of mRNAs (6, 7). β mRNAs are detected soon after the synthesis of α proteins. Many β proteins are involved in the process of viral DNA replication. The expression of β genes occurs at maximal rates following the initiation of DNA replication. The inhibition of viral DNA replication causes a moderate reduction in the accumulation of γ_1 mRNAs and a strong reduction in the accumulation of γ_2 mRNAs (8).

To date, only a few HSV regulatory proteins have been identified. The αICP4 protein appears to exert positive and negative effects on HSV gene expression. At the nonpermissive temperature (NPT), temperature-sensitive mutants defective in ICP4 overproduce α mRNAs and proteins but fail to induce the expression of β, γ_1, and γ_2 mRNAs and proteins (9–13). The αICP4 and αICP0 proteins can induce the expression of β genes introduced into cells by transfection (14–16), but it is not known if ICP0 is involved in the induction of HSV gene expression during a lytic infection. Defects in the βICP8 protein result in the increased accumulation of mRNAs from genes of the α, β, γ_1, and γ_2 classes (17, 18). There is evidence that a component of the HSV virion, possibly VP16, stimulates the expression of α genes (19–23). Another virion component negatively regulates the translation of host and viral α mRNAs (24). The mechanism of action of these regulatory proteins has not been determined.

The regulation of chimeric genes consisting of a promoter of an α or a γ_2 gene linked to the structural sequences for the β thymidine kinase gene reflects the temporal class of the promoter (22, 25). This implies that transcriptional regulation plays a major role in the turn-on of HSV gene expression. To further examine the control of viral gene expression, we have measured the level of transcription of the *αICP4*, *βICP8*, *γ_1ICP5*, and γ_2 glycoprotein C (*gC*) genes at various times during a lytic infection. We have also analyzed the effect of inhibition of viral DNA replication and of defects in the ICP4 or ICP8 protein on the transcription of these genes. Our results support the model that transcriptional regulation is an important mechanism for the control of HSV gene expression. We have also identified a β gene product that is required for the shutoff of transcription of the *αICP4* gene.

MATERIALS AND METHODS

Cells and Viruses. Procedures used for propagating and titering viral stocks have been described (17, 26). The HSV-1 strains KOS1.1, KOS1.1*ts*756 (27), and KOS1.1*ts*18 (28) were provided by M. Levine (University of Michigan, Ann Arbor). Phosphonoacetic acid was used at a concentration of 400 μg/ml, which totally inhibits viral DNA replication (18).

Construction of Recombinant M13 Phage and Isolation of Single-Stranded (ss) Virion DNA. A series of M13 clones was constructed such that the ss virion DNA contained HSV DNA inserts that were complementary (c) or anticomplementary (a) to the mRNAs encoding ICP4, ICP5, ICP8, or gC. These clones contained the indicated HSV sequences inserted into the *Bam*HI site of mp19: 5′ αICP4-a and 5′ αICP4-c, *Bam*HI Y [ref. 21, map coordinates 0.853–0.865, *ca.* 1.75 kilobases (kb)]; 3′ αICP4-a and 3′ αICP4-c, *Sau*3A B (ref. 29, 0.835–0.845, *ca.* 1.5 kb); γ_1ICP5-a and γ_1ICP5-c, *Bam*HI A′ (ref. 30, 0.255–0.266, *ca.* 1.5 kb). βICP8-a and βICP8-c contained a *Kpn* I–*Bam*HI fragment (ref. 28, 0.398–0.407, *ca.* 1.4 kb) inserted into the *Kpn* I and *Bam*HI sites of mp18 and mp19, respectively. γ_2gC-a and γ_2gC-c contained an *Eco*RI–*Xba* I fragment (ref. 31, 0.633–0.639, *ca.* 0.9 kb) inserted into the *Eco*RI and *Xba* I sites of mp18 and mp19, respectively. The identity and orientations of these fragments were confirmed by secondary restriction enzyme cuts and in some cases by DNA sequencing. In addition, these clones hybridized to mRNAs of the expected size when used as probes on RNA transfer blots containing total infected-cell RNA (results not shown).

ss M13 DNAs were isolated by phenol and chloroform extraction of virions that had been purified by isopycnic centrifugation in CsCl (32).

Measurement of Transcription. Vero cells (2×10^7) were infected at a multiplicity of infection (moi) = 20. Procedures for the isolation of nuclei, the run-off transcription assay, the

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Abbreviations: ICP, infected cell polypeptide; gC, glycoprotein C; HSV, herpes simplex virus; NPT, nonpermissive temperature; kb, kilobase(s); ss, single-stranded.

isolation of nuclear RNA, and conditions of hybridization were performed essentially as described (33), except that the hybridization was for 48 hr at 66°C. Identical amounts of trichloroacetic acid-precipitable ^{32}P radioactivity for each sample in an experiment were added to each hybridization reaction. The incorporation of trichloroacetic acid-precipitable radioactivity in nuclei was linear during the course of the run-off reaction. We typically isolated 1–2 × 10^7 cpm per 2 × 10^7 cells. The amount of trichloroacetic acid-precipitable radioactivity recovered from all samples within a particular experiment did not vary by >30%. At late times (>8 hr after infection) we found that total incorporation of ^{32}P dropped to <50% of the amount from mock-infected cells; therefore, we did not extend our analysis beyond 8 hr after infection. The filters were washed twice in 2× concentrated SET buffer/0.1% $NaDodSO_4$ (SET buffer = 0.15 M NaCl/20 mM Tris·HCl, pH 7.4/1 mM EDTA) at room temperature for 30 min and then were washed twice in 0.1× SET buffer/0.1% $NaDodSO_4$ at 60°C for 30 min. The filters then were incubated at room temperature in 2× SET buffer/0.1% $NaDodSO_4$/5 μg of RNase A per ml for 30 min and then were washed twice in 0.1× SET buffer/0.1% $NaDodSO_4$ at 60°C. The filters were exposed to preflashed Kodak XRP-1 film at −70°C with an intensifying screen.

The amount of hybridization to the probes was determined by microdensitometry (26). The amount of transcription is expressed in arbitrary units, corrected for the length of the HSV DNA. No correction was made for the diploid nature of the *ICP4* genes.

To prepare filters for hybridization, ss M13 DNAs were bound to nitrocellulose in 0.9 M NaCl/90 mM sodium citrate, pH 7, by using the Schleicher & Schuell slot blot apparatus. Ten micrograms of DNA was applied per slot. Control experiments showed that the conditions for hybridization and washing were reproducible. Virtually identical patterns of hybridization were observed when multiple filters were incubated with labeled RNA isolated from HSV-infected cells. No hybridization was observed with labeled RNA from mock-infected cells.

RESULTS

Nuclear run-off assays have been used extensively to measure the levels of transcription of eukaryotic genes (33–35). To measure the transcription of HSV genes, we isolated nuclei from cells infected with the wild-type or mutant virus. Nascent transcripts were elongated by incubation of the nuclei with exogenous nucleoside triphosphates and [α-^{32}P]UTP. Total RNA was purified and hybridized to ss recombinant M13 DNAs that had been blotted on nitrocellulose filters. These ss DNAs contained sequences from the 5′ or 3′ portion of the *αICP4* gene or sequences from the *βICP8*, *γ₁ICP5*, or *γ₂gC* gene. The orientation of the HSV DNA insert with respect to the M13 sequences was such that ss M13 virion DNAs contained HSV DNA sequences complementary or anticomplementary to HSV mRNAs. The measurement of transcription of each strand within a gene was necessitated by the observation that at least 55% of the HSV genome is transcribed symmetrically at late times (36).

Effect of Viral DNA Replication on HSV Transcription. We first examined the transcription of viral genes as a function of viral DNA replication. Cells were infected with the wild-type strain HSV-1 KOS1.1 in the presence or absence of phosphonoacetic acid, a specific inhibitor of the viral DNA polymerase (37). Transcription was assayed in nuclei prepared at 2, 4, 6, or 8 hr after infection. The results of this experiment are shown in Fig. 1. Quantitation of the amount of hybridization to the probes is shown in Figs. 2 and 3. In cells treated with phosphonoacetic acid (Fig. 1*A*), transcription of the 5′ and 3′ portions of the *ICP4* gene (detected with

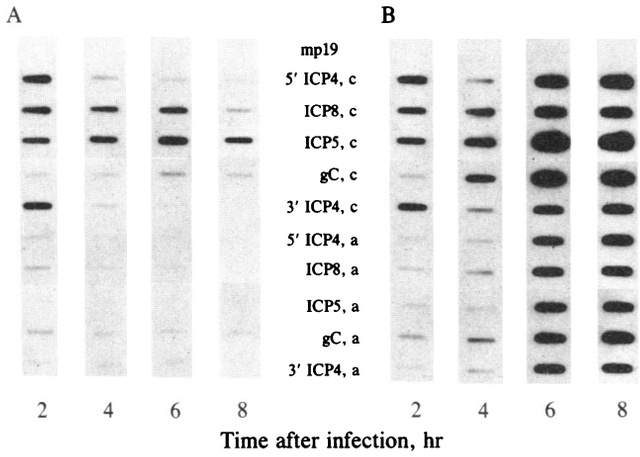

FIG. 1. Time course of transcription of HSV genes in the presence or absence of phosphonoacetic acid, an inhibitor of viral DNA replication. At the indicated times, nuclei were isolated from cells infected with HSV-1 in the presence (*A*) or absence (*B*) of phosphonoacetic acid. Radioactively labeled run-off transcripts synthesized in isolated nuclei were hybridized to ss M13 DNAs that had been blotted onto nitrocellulose filters. The ss DNAs contained either no insert (mp19) or HSV sequences complementary (c) or anticomplementary (a) to the mRNAs for the 5′ or 3′ region of the *ICP4* (5′ ICP4 and 3′ ICP4, respectively), *ICP8*, *ICP5*, or *gC* genes. The film was preflashed and exposed at −70°C for 24 hr with an intensifying screen.

the 5′ and 3′ αICP4-c probes, respectively) was similar. Transcription was greatest at 2 hr after infection and appeared to shut off by 4 hr after infection (Fig. 2 *A* and *B*). In this experiment, transcription of the *ICP8* gene (detected with βICP8-c) was already near its peak level by 2 hr after infection (Fig. 2*C*). By 8 hr after infection, βICP8-c transcription dropped to 30% of its peak level (6 hr after

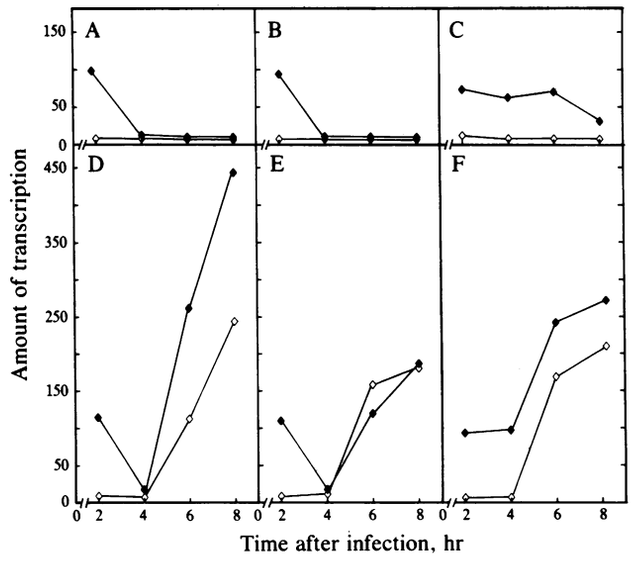

FIG. 2. Quantitative comparison of the transcription of the *ICP4* and *ICP8* genes in the presence or absence of phosphonoacetic acid. The intensities of the bands from the experiment shown in Fig. 1 were determined by microdensitometry of a series of autoradiographs. The level of transcription (after subtracting background hybridization to the vector DNA and normalizing for the length of the HSV inserts) of the different genes was expressed in arbitrary units. Hybridization to a HSV insert complementary (◆) or anticomplementary (◇) to the 5′ region of *ICP4* (*A* and *D*), the 3′ region of *ICP4* (*B* and *E*), and *ICP8* (*C* and *F*). Nuclei were isolated from cells infected in the presence (*A–C*) or absence (*D–F*) of phosphonoacetic acid.

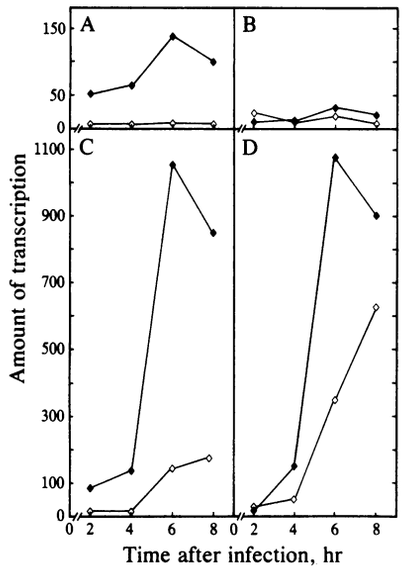

FIG. 3. Quantitative comparison of the transcription of the *ICP5* and *gC* genes in the presence or absence of phosphonoacetic acid. The amount of transcription was determined as in Fig. 2. Hybridization to a HSV insert complementary (◆) or anticomplementary (◇) to ICP5 (*A* and *C*) or gC (*B* and *D*) mRNA. Nuclei were isolated from cells infected in the presence (*A* and *B*) or absence (*C* and *D*) of phosphonoacetic acid.

infection). Transcription of γ_1ICP5-c increased until 6 hr after infection and then dropped slightly by 8 hr after infection (Fig. 3*A*). Very low amounts of γ_2gC-c transcription were observed (Fig. 3*B*). In the absence of viral DNA replication, transcription of these genes was strongly asymmetric in that very little hybridization to the a strands was observed.

In cells not treated with phosphonoacetic acid (Fig. 1*B*), transcription of 5′ αICP4-c, 3′ αICP4-c, and βICP8-c at 2 and 4 hr after infection was similar to that observed in phosphonoacetic acid-treated cells—i.e., transcription of the *ICP4* genes appeared to shut off while transcription of the *ICP8* gene remained relatively constant (Fig. 2 *D–F*). At 6 and 8 hr after infection, the amount of transcription detected with the a and c strands of 5′ αICP4, 3′ αICP4, and βICP8 increased significantly. The amount of transcription detected with the γ_1ICP5-c and γ_2gC-c probes at peak times (6 hr after infection) was *ca.* 10- and 30-fold greater, respectively, than the peak levels observed in phosphonoacetic acid-treated cells (Fig. 3). Significant transcription of the a strands was also observed. Because there are several explanations for this symmetric transcription (see *Discussion*), we have restricted further analysis to transcription under early conditions.

These experiments show that the transcription of the *ICP4* genes is shut off by 4 hr after infection in cells treated with phosphonoacetic acid. In untreated cells, *ICP4* gene transcription was also shut off at 4 hr after infection. In addition, the transcription of the γ_2*gC* gene was strongly inhibited when DNA replication was blocked by phosphonoacetic acid.

It is worth noting that the time course of transcription of these genes seemed to vary, depending on the physiology of the host cells. In other experiments using cells at a higher passage number, the viral infection appeared to proceed more slowly. For example, transcription of the *ICP8* and *ICP5* genes was barely detectable at 2 hr after infection, whereas *ICP4* gene transcription was detectable at very high amounts (data not shown).

Effect of a ICP4 Defect on Transcription. Although current experimental evidence strongly suggests that ICP4 acts at the transcriptional level to control gene expression (1, 2, 9–16, 29), the effect of a defect in ICP4 on the transcription of HSV genes has not been tested by using short labeling periods. To directly test the hypothesis that ICP4 is required for the transcription of viral β, γ_1, and γ_2 genes, we analyzed the transcription of HSV genes in cells infected with the temperature-sensitive ICP4 mutant KOS1.1*ts*756 (27). This mutant is similar to other complementation group 1–2 members in that cells infected with *ts*756 synthesize primarily α proteins at the NPT (unpublished results). Cells were infected with *ts*756 or *ts*$^+$ virus at the NPT, 39.5°C, and transcription was measured in nuclei prepared at 1.5, 3, or 5 hr after infection (Fig. 4). At 1.5 and 3 hr after infection, transcription detected with the 5′ αICP4-c probe was greater in cells infected with the ICP4 mutant. Similar results were obtained by using the 3′ αICP4 probe (results not shown). Greatly reduced amounts of hybridization to the βICP8-c, γ_1ICP5-c, and γ_2gC-c probes were detected in cells infected with the ICP4 mutant. At the permissive temperature, transcription of these genes was similar in cells infected with *ts*756 or *ts*$^+$ virus (results not shown). These results prove that ICP4 is required for the transcription of β, γ_1 and γ_2 mRNAs. It is interesting that the amount of *αICP4-c* transcription appears to decline from 1.5 to 5 hr after infection with *ts*756. The significance of this observation will be discussed below.

Effect of a ICP8 Defect on Viral Transcription. The major DNA-binding protein of HSV, ICP8, appears to be multifunctional. Temperature-sensitive mutants defective in this protein fail to replicate their DNA at the NPT (28, 38). Under conditions that inhibit viral DNA replication, cells infected with a ICP8 mutant virus accumulate increased levels of the mRNAs encoding ICP4, ICP8, ICP5, and gC at the NPT. The accumulation of these mRNAs is normal at the permissive temperature (17, 18). These studies suggested that ICP8 affected either the transcription or stability of these mRNAs. Therefore, we compared the transcription of these genes in cells infected with ICP8 mutant virus KOS1.1*ts*18 or *ts*$^+$ virus (28). Cells were infected at the NPT in the presence of phosphonoacetic acid, and transcription was measured in nuclei prepared at 3 or 6 hr after infection. As observed earlier, transcription of the *ICP4* gene was detected at early times (3 hr after infection) with the wild-type virus but was

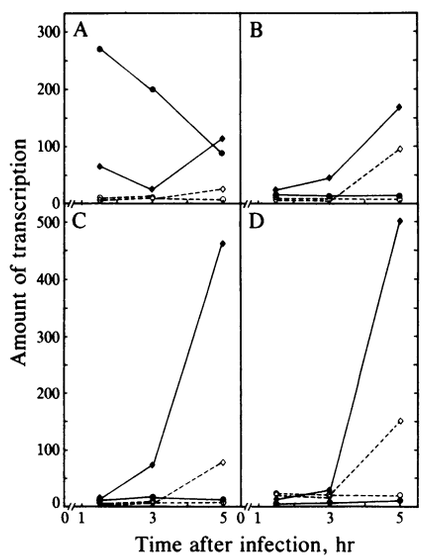

FIG. 4. Effect of a defect in ICP4 on the transcription of HSV genes. The amount of transcription of the *ICP4 (A), ICP8 (B), ICP5 (C),* and *gC (D)* genes in nuclei from cells infected at 39.5°C with *ts*756 (●, ○) or the *ts*$^+$ (◆, ◇) virus was determined as described in the legends of Figs. 1 and 2. Closed symbols represent hybridization to the c probes; open symbols represent hybridization to the a probes.

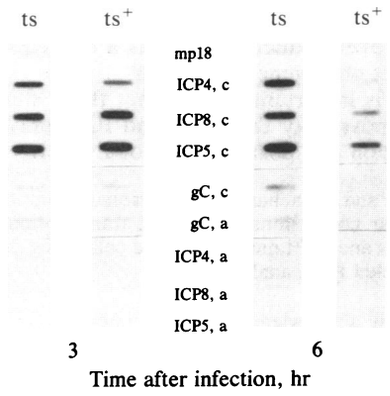

FIG. 5. Effect of a defect in ICP8 on the transcription of HSV genes. Nuclear run-off transcripts from cells infected for 3 or 6 hr at 39.5°C with the ICP8 mutant KOS1.1*ts*18 (ts) or the *ts*$^+$ virus (ts$^+$) were hybridized to the indicated ss M13 DNAs. Transcription of the *ICP4* genes was measured with the 5′ ICP4-c and -a probes.

shut off at late times (6 hr after infection) (Fig. 5). In cells infected with the ICP8 mutant, transcription of ICP4-c was *ca.* 2- to 3-fold greater at 3 hr after infection and then remained at a high level until at least 6 hr after infection. Quantitation of a similar experiment is shown in Fig. 6. These results show that the transcription of αICP4-c fails to shut off in cells infected with an ICP8 mutant. Transcription of βICP8-c, γ_1ICP5-c, and γ_2gC-c was similar in cells infected with either the *ts*$^+$ or *ts*18 virus at 3 hr after infection. By 6 hr after infection, the transcription of these genes had decreased in cells infected with the *ts*$^+$ virus. The earlier shutoff of these genes at 39.5°C compared to that observed at 37°C (Fig. 2) probably reflected a more rapid infection occurring at this temperature. Transcription of βICP8-c, γ_1ICP5-c, and γ_2gC-c was detected at high levels at 6 hr after infection in cells infected with the ICP8 mutant at the NPT (Fig. 5). At the permissive temperature, the level of transcription of these genes was similar in cells infected with *ts*18 or *ts*$^+$ virus (results not shown). These results show that ICP8 is required for the shutoff of *ICP4* gene transcription and may

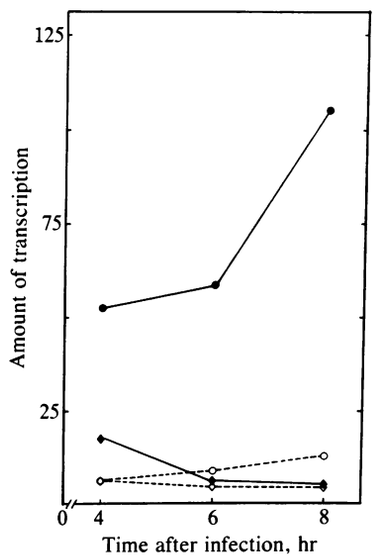

FIG. 6. Time course of transcription of the *ICP4* genes in cells infected with a ICP8 mutant. The amount of transcription homologous to the 3′ ICP4-c (closed symbols) or -a (open symbols) probe at various times in cells infected with *ts*18 (●, ○) or the *ts*$^+$ (◆, ◇) virus was determined as in Fig. 2. Similar results were obtained when transcription of the *ICP4* genes was measured with the 5′ ICP4 probe.

function to negatively regulate the transcription of β, γ_1, and γ_2 genes from parental genomes.

DISCUSSION

We have used a nuclear run-off transcription assay to analyze the expression of an α, a β, a γ_1, and a γ_2 gene. The major conclusions of this work are as follows. (*i*) Transcription of the *ICP4* genes is efficiently shut off by 4 hr after infection. (*ii*) The negative regulation of *ICP4* gene transcription requires the β gene product, ICP8. (*iii*) The failure to accumulate β and γ mRNAs in cells infected with a ICP4 mutant results from a lack of transcription of these genes. (*iv*) The transcription of γ_2 genes is almost totally inhibited in the absence of DNA replication. (*v*) Defects in ICP8 result in increased transcription of the *ICP8*, *ICP5*, and *gC* genes from parental genomes.

Control of αICP4 Gene Transcription. The transcription of the *ICP4* genes, as well as other α genes, is stimulated by a component of the HSV virion (19–23). αICP4-c transcription was detected at high levels at the earliest time points examined in this study (1.5 hr after infection). By 4 hr after infection, transcription of αICP4-c had shut off. Thus, *ICP4* gene transcription is positively and negatively regulated. The negative regulation probably requires ICP8 but may also involve ICP4, because defects in either of these proteins resulted in increased transcription of the *ICP4* gene. However, the lack of shutoff of *ICP4* gene transcription in cells infected with a ICP4 mutant may be due to the failure to induce normal levels of ICP8. Our results indicate that the shutoff of *ICP4* gene transcription is not simply a consequence of α genes modifying the transcriptional machinery such that only β and γ genes are transcribed. In fact, defects in ICP8 result in increased transcription of all classes of genes from parental genomes.

Following the initial shutoff of *ICP4* transcription, we detected a resumption of transcription of the *ICP4* genes when DNA replication was not inhibited. We are uncertain of the signifjcance of this observation because we detect high levels of transcription from the c and a strands of our probes at late times. Several explanations for our data exist. First, transcription could result from readthrough of termination signals at late times (2). We detect a transcript of *ca.* 8 kb at late times that is homologous to the 5′ and 3′ αICP4-c probes (data not shown). Second, much of the late transcription could represent aberrant transcription of progeny DNA molecules. This may or may not be an *in vitro* artifact. Third, it is possible that there is spurious hybridization to a (G + C)-rich transcript synthesized only when DNA replication is not inhibited. Thus, although it is possible that authentic transcription of the *ICP4* gene reinitiates at late times, other explanations cannot be ruled out.

Control of βICP8 Gene Transcription. Transcription of the *ICP8* gene can be detected at high levels as early as 2 hr after infection and is greatly facilitated by ICP4. When DNA replication was inhibited, transcription remained at about the same level from 2 to 6 hr after infection and then dropped to about 30% of the peak value by 8 hr after infection. The results reported here indicate that the transcription of the *ICP8* gene from parental genomes is inhibited directly or indirectly by its gene product.

If viral DNA replication is not inhibited, the transcription of the *ICP8* gene continues to increase from 4 to 8 hr after infection. Transcription detected with the βICP8-a probe increases in parallel. We do detect slightly increased levels of ICP8 mRNA at late times if DNA replication is not inhibited (unpublished results). Also, a minor 10-kb transcript is transcribed in the same direction as the ICP8 mRNA (refs. 28 and 39; unpublished results). We speculate that the apparent increase in transcription of the *ICP8* gene at late times results in part from readthrough transcripts.

The α protein ICP0 appears to be able to stimulate the expression of *ICP8* and other β genes when plasmids containing these genes are introduced into cells (14–16). The results reported here show that other α genes do not induce significant levels of transcription of *ICP8* in cells infected with an ICP4 mutant at the NPT. It is interesting to note that nuclear transport of ICP0 is defective in cells infected with *ts*756 at the NPT (unpublished data). Thus, a defect in ICP4 may exhibit *trans*-dominant effects on the transport of other proteins.

Transcription of the γ_1*ICP5* and γ_2*gC* Genes. Transcription of the *ICP5* gene and *gC* gene is dependent on functional ICP4. When DNA replication was inhibited, transcription of γ_1ICP5-c increased from 2 to 6 hr after infection and then decreased slightly. The transcription of γ_2gC-c was barely detectable in the absence of viral DNA replication. This confirms the results of Silver and Roizman (25), which indicate that the failure to accumulate γ_2 transcripts in the absence of DNA replication is due to the inhibition of their transcription. The peak level of transcription of γ_1ICP5-c and γ_2gC-c was increased about 10- and 30-fold, respectively, when viral DNA replication was allowed to proceed. The amount of hybridization to these probes was considerably greater than to the γ_1ICP5-a, γ_2gC-a, βICP8-a or -c, or αICP4-a or -c probe. These results support the hypothesis that viral DNA replication is required for full-level transcription of these genes. However, the absolute magnitude of the effect of DNA replication on the specific transcription of these genes is difficult to judge because of the possible contribution of readthrough transcripts at late times.

Role of ICP4 and ICP8 in the Regulation of Viral Gene Transcription. At early times, the ICP4 mutant *ts*756 transcribes the *ICP4* genes at increased rates and fails to induce transcription at normal levels of the *ICP8*, *ICP5*, and *gC* genes. These results confirm previous conclusions that ICP4 is required for the negative regulation of *αICP4-c* transcription and the positive regulation of β, γ_1, and γ_2 gene transcription. It is interesting that the amount of *ICP4* gene transcription appears to decline from 1.5 to 5 hr after infection with *ts*756. There are several possible explanations for this. (*i*) A toxic effect of the mutant polypeptide may exist, perhaps by destabilizing cellular or viral factors required for the transcription of the *ICP4* gene (19–23). (*ii*) Sufficient activity of the ts ICP4 may remain to negatively regulate *ICP4* gene transcription either directly or indirectly by allowing low-level expression of other gene products—i.e., ICP8. We do detect small amounts of *ICP8* gene transcription (Fig. 4) and ICP8 protein (unpublished) in cells infected with *ts*756 at the NPT. (*iii*) Other viral or cellular gene products might inhibit *ICP4* gene transcription under these conditions.

A defect in the β protein ICP8 results in the continued transcription of αICP4-c and an increase in transcription of βICP8-c, γ_1ICP5-c, and γ_2gC-c. These results indicate that ICP8 is required for the shutoff of *ICP4* gene transcription and a decrease in the transcription of β, γ_1, and γ_2 genes from parental genomes. ICP8 binds double-stranded (ds) and ss DNA. The affinity of ICP8 for ss DNA is greater than the affinity for ds DNA, and the ss and ds DNA-binding activities are probably distinct (40–42). Sequence-specific binding of ICP8 for viral DNA has not yet been observed *in vitro*. ICP8 could control transcription by coating ds viral DNA in a nonspecific manner. However, it is still possible that ICP8 recognizes specific sequences or DNA–protein complexes *in vivo*.

Many questions remain to be answered. (*i*) Is the action of ICP4 or ICP8 in the negative regulation of *ICP4* gene transcription direct or indirect? (*ii*) Do ICP8 and ICP4 negatively regulate *ICP4* gene transcription by a common mechanism? (*iii*) Does ICP8 affect the transcription of genes from different kinetic classes by similar mechanisms or are β and γ genes overproduced simply as a consequence of the failure to shut off α gene expression?

Finally, it is important to note that these studies do not exclude the possibility that ICP4 and ICP8 may also affect gene expression at a posttranscriptional level.

Note. D. Yager and S. Bachenheimer (personal communication) have reached similar conclusions concerning transcriptional control in wild-type virus and ICP4 mutant-infected cells using *in vitro* run-off and pulse-labeled RNA analyses.

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