The Role of Herpes Simplex Virus ICP27 in the Regulation of *UL24* Gene Expression by Differential Polyadenylation

LOUANE E. HANN,[1] W. JAMES COOK,[1]† SUSAN L. UPRICHARD,[2,3]
DAVID M. KNIPE,[2,3] AND DONALD M. COEN[1,3]*

*Departments of Biological Chemistry and Molecular Pharmacology[1] and Microbiology
and Molecular Genetics[2] and Committee on Virology,[3] Harvard
Medical School, Boston, Massachusetts 02115*

Herpes simplex virus specifies two sets of transcripts from the *UL24* gene, short transcripts (e.g., 1.4 kb), processed at the *UL24* poly(A) site, and long transcripts (e.g., 5.6 kb), processed at the *UL26* poly(A) site. The 1.4- and 5.6-kb transcripts initiate from the same promoter but are expressed with early and late kinetics, respectively. Measurements of transcript levels following actinomycin D treatment of infected cells revealed that the 1.4- and 5.6-kb *UL24* transcripts have similar stabilities, consistent with *UL24* transcript kinetics being regulated by differential polyadenylation rather than by differential stabilities. Although the *UL24* poly(A) site, which gives rise to short transcripts, is encountered first during processing, long transcripts processed at the *UL26* site are equally or more abundant; thus, operationally, the *UL24* site is weak. Using a series of viral *ICP27* mutants, we investigated whether ICP27, which has been suggested to stimulate the usage of weak poly(A) sites, stimulates 1.4-kb transcript accumulation. We found that accumulation of 1.4-kb transcripts did not require ICP27 during viral infection. Rather, ICP27 was required for full expression of 5.6-kb transcripts, and the decrease in 5.6-kb transcripts relative to 1.4-kb transcripts was not due solely to reduced DNA synthesis. Our results indicate that temporal expression of *UL24* transcripts can be regulated by differential polyadenylation and that although ICP27 is not required for processing at the operationally weak *UL24* poly(A) site, it does modulate 5.6-kb transcript levels at a step subsequent to transcriptional initiation.

During viral infection, transcriptional regulation plays a major role in maintaining the coordinated expression of viral genes. However, other regulatory mechanisms, such as those that exert their effects posttranscriptionally, are important in the viral life cycle. Examples include translational control (17, 25, 58, 68), RNA editing (3, 22, 65), RNA transport (8, 12), RNA stability (24, 59), splicing (12), and 3'-end formation and polyadenylation (11, 40). However, many of the *cis*- and *trans*-regulatory elements that govern posttranscriptional control during viral infection remain uncharacterized.

We have been studying differential polyadenylation of the herpes simplex virus type 1 (HSV-1) *UL24* gene. *UL24* transcripts possess two distinct 3' ends due to the alternative use of two poly(A) sites (6). One site is promoter proximal, located 191 nucleotides downstream of the *UL24* termination codon (nucleotide 48737, using the numbering of McGeoch et al. [34]), whereas the other is located roughly 4.0 kb downstream, 49 nucleotides 3' of the *UL26* open reading frame (ORF) (6) (Fig. 1). As three different 5' ends have been detected for *UL24* transcripts (Fig. 1) (26, 45, 46, 67), the *UL24* locus gives rise to six different mRNAs. For example, the 1.4- and 5.6-kb *UL24* transcripts initiate at or near nucleotide 47402 but have different 3' termini due to processing at the *UL24* and *UL26* poly(A) sites, respectively (6). It is usually the rule that transcripts are processed at the promoter-proximal poly(A) site (9, 11, 29, 66) because 3' processing is coupled with transcription and occurs on nascent transcripts (33). Only when the promoter-proximal polyadenylation signal is weak does processing occur at downstream poly(A) sites (11, 66). The observation that 5.6-kb *UL24* transcripts, processed at the promoter-distal *UL26* poly(A) site, are as abundant as or more abundant than 1.4-kb transcripts (6) processed at the promoter-proximal *UL24* poly(A) site indicates that the *UL24* poly(A) site is operationally weak. Interestingly, the patterns of temporal expression of these two transcripts are different, with the 1.4-kb transcript displaying early gene kinetics and the 5.6-kb transcript exhibiting leaky-late gene kinetics (6). One explanation for the different kinetics displayed by the two transcripts is that the 1.4-kb *UL24* transcript decays more rapidly than the 5.6-kb transcript. However, an alternative explanation is that the temporal expression of these two transcripts is modulated in part by differential polyadenylation.

Previous studies have suggested that the HSV regulatory protein ICP27 (also known as IE63, IE2, and UL54) stimulates 3'-end processing of genes containing nonoptimal or weak polyadenylation signals, at least in transfected cells and in in vitro polyadenylation reactions (35, 57). ICP27 is required for lytic infection and efficient viral DNA replication (32, 48, 52). It can repress the expression of several immediate-early and early genes (1, 10, 19, 38, 47, 50, 62) and can *trans* activate viral gene expression, as evidenced by the requirement of ICP27 for early and late gene expression (21, 32, 35, 48, 52, 64). Other roles proposed for ICP27 include inhibition of host cell pre-mRNA splicing (18, 20, 41, 43, 56, 57, 60), stimulation of late gene transcription (28) and posttranscriptional processes (60), and export of late gene mRNAs from the nucleus to the cytoplasm (42, 61).

In this study, we examined the stabilities of the *UL24* transcripts and the role of ICP27 in expression of these transcripts. We hypothesized that ICP27, which has been reported to stimulate weak polyadenylation signal usage (57), might play a role

---

* Corresponding author. Mailing address: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 250 Longwood Ave., Boston, MA 02115. Phone: (617) 432-1691. Fax: (617) 432-3833. E-mail: dcoen@warren.med.harvard.edu.

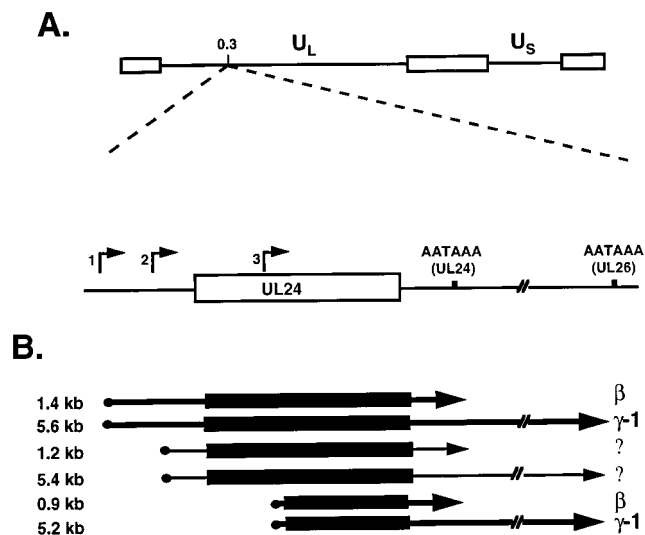† Present address: Millennium Pharmaceuticals, Cambridge, MA 02139.

A.

B.

FIG. 1. The mRNAs transcribed from the *UL24* locus. (A) Schematic representation of the HSV-1 genome (prototype arrangement) indicating the location of the *UL24* gene at map coordinate 0.3. The major repeat sequences are represented by the open rectangles that flank the unique long (U_L) and unique short (U_S) regions as shown. The *UL24* locus is diagrammed below the HSV-1 genome. The three *UL24* promoters are indicated by arrows numbered 1, 2, and 3 and are located at nucleotides 47402, 47666, and 48076 relative to the HSV-1 strain 17 sequence (34). Positions of the *UL24* ORF (open rectangle) and of the *UL24* and *UL26* poly(A) sites are shown. (B) Locations of the six mRNAs transcribed from the *UL24* locus. Solid circles represent the 5′ cap, thin lines represent the 5′ and 3′ UTRs, the solid box represents the *UL24* ORF, and the poly(A) tail is indicated by arrowheads. The thicker lines in the 1.4-, 5.6-, 0.9-, and 5.2-kb transcripts are reflective of the strengths of promoters 1 and 3, which are stronger than promoter 2. The molecular lengths of the transcripts are indicated to the left of the transcripts; the temporal gene classes for the 1.4-, 5.6-, 0.9-, and 5.2-kb transcripts are indicated at the right; the temporal gene class for the 5.4- and 1.2-kb transcripts has not been determined conclusively due to their low abundance.

in stimulating processing at the *UL24* poly(A) site early in infection. We found that the 1.4- and 5.6-kb transcripts have similar stabilities in infected cells. Contrary to our expectations, we found that an *ICP27* null mutant expressed higher levels of the 1.4-kb transcript than of the 5.6-kb transcript. These results provide evidence that alternative polyadenylation site usage is in part responsible for the temporal expression of the two transcripts and that although usage of the operationally weak 1.4-kb *UL24* poly(A) site does not require ICP27 in infected cells, ICP27 acts at a step subsequent to transcriptional initiation, leading to an increase in the levels of the 5.6-kb transcript.

MATERIALS AND METHODS

Viruses and cells. The wild-type (wt) HSV-1 strain used in this study was KOS1.1 (23), which was propagated in Vero cells (American Type Culture Collection, Rockville, Md.) as described previously (4). *ICP27* mutant viruses *d27*, *n504R*, and *n263R* (48, 50) were propagated in the *ICP27*-complementing cell line V27 (48). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 5% newborn calf serum, 10 mM HEPES (pH 7.5), penicillin-streptomycin (100 U/ml), and 200 mM L-glutamine. For studies of mRNA stability, Dulbecco's modified Eagle's medium was replaced at 5 h postinfection with medium containing actinomycin D (5 μg/ml; Sigma Chemical, St. Louis, Mo.). Where indicated, viral DNA replication was inhibited by the addition of sodium phosphonoacetate (PAA; Sigma) to the medium at a concentration of 400 μg/ml at the time of virus adsorption.

RNA isolation and Northern blot analysis. Total RNA was prepared from infected cells by using an RNAqueous kit (Ambion, Inc., Austin, Tex.) or as described previously (7). Cytoplasmic RNA was prepared as described previously (64). Polyadenylated mRNA was selected from 10 μg of total or cytoplasmic RNA, using a MicroPoly(A)Pure mRNA purification kit (Ambion) according to the manufacturer's instructions except that the RNA pellet was washed twice

with 70% ethanol, air dried briefly, and resuspended in sample buffer (10 mM morpholinepropanesulfonic acid [MOPS; pH 7.0], 4 mM sodium acetate, 0.5 mM EDTA, 50% freshly thawed formamide, 6% formaldehyde, 7% glycerol). Samples were heated at 68°C for 15 min, placed immediately on ice, and then separated by electrophoresis into a 1% denaturing agarose formaldehyde gel (SeaKem LE agarose; FMC BioProducts, Rockland, Maine) at 115 V in 10 mM MOPS (pH 7.0)–4.0 mM sodium acetate–0.5 mM EDTA (55). RNA was transferred overnight to Duralose membranes (Stratagene) in 20× SSC (3.0 M NaCl, 0.3 M sodium citrate [pH 7.0]) and was cross-linked to the membrane by using UV light. Northern blot hybridization and probe preparation were performed essentially as described previously (15). The *UL24* probe, a 550-bp *Eco*RI-*Hind*III fragment that does not contain *tk* sequences, was isolated from pJC2. pJC2 was made by digesting pLS/ts+5/+15 (5) with *Eco*RI and *Hind*III and ligating the purified 550-bp fragment into *Eco*RI- and *Hind*III-digested pBluescriptII KS(+) (Stratagene, La Jolla, Calif.). All blots were stripped and re-probed for *gB* and *ICP8*. The probe for *gB* transcripts was a 900-bp *Pst*I fragment from pSG18:SalE (44). The *ICP8* probe was a 1.4-kb *Pst*I-*Pst*I fragment from plasmid pJC8, which was constructed by digesting pSG18Sal I-A (27) with *Pst*I and ligating the 1.4-kb *Pst*I-*Pst*I fragment into *Pst*I-digested pBluescriptII KS(+). Hybridized blots were exposed to Kodak X-ray film, and the resulting autoradiograms were scanned by using ScanWizard and Adobe Photoshop. Quantification was performed with NIH Image.

RESULTS

Stabilities of the 1.4- and 5.6-kb *UL24* transcripts. Previous data suggested that the temporal expression of *UL24* transcripts was regulated by differential polyadenylation (6). Short transcripts (e.g., 1.4 kb), utilizing the *UL24* poly(A) site, are expressed maximally at early times in infection and then decrease, whereas long transcripts (e.g., 5.6 kb), processed at the *UL26* poly(A) site, continue to accumulate throughout infection. However, another interpretation of the data was that the shorter transcripts are inherently less stable than the longer transcripts. To address these alternative hypotheses, we assessed the stabilities of the 1.4- and 5.6-kb transcripts. Vero cells were infected with HSV-1 KOS1.1 (wt) at 10 PFU/cell. At 5 h postinfection, further RNA synthesis was inhibited by addition of actinomycin D (5 μg/ml). Total RNA was isolated at 0.5-h intervals, poly(A) selected, and analyzed by Northern blot hybridization with a *UL24*-specific probe. Representative data are shown in Fig. 2. Quantification of the 1.4- and 5.6-kb bands showed that the relative levels of the two transcripts were similar at all time points following actinomycin D addition, indicating that the decay rates were similar. Additionally, the relative levels of the 0.9- and 5.2-kb *UL24* transcripts that initiate at or near nucleotide 48076 (6) were similar following actinomycin D treatment (Fig. 2A), indicating that the stabilities of these transcripts were not significantly different. These data are consistent with the idea that the different kinetics of expression of the short and long *UL24* transcripts are due to differential usage of the *UL24* and *UL26* poly(A) sites and not due to different decay rates.

Infection of cells with *ICP27* mutants has little effect on 1.4-kb transcript levels but causes a reduction in 5.6-kb transcript levels. During transcription of the *UL24* locus, the *UL24* poly(A) site is encountered first by the cellular transcriptional/processing machinery and thus has a major advantage over the more distal *UL26* poly(A) site (11, 66). However, long *UL24* transcripts, which are processed at the *UL26* site, are as abundant as or more abundant than those that initiate at the same promoter and utilize the *UL24* poly(A) site. Thus, based on the abundance of short transcripts relative to long transcripts in virus-infected cells, the *UL24* polyadenylation signal is operationally weak. Previous studies using transfected plasmids and in vitro polyadenylation reactions suggested that the essential regulatory HSV-1 gene, *ICP27*, stimulates usage of weak polyadenylation signals (35, 57). Thus, we hypothesized that ICP27 might stimulate the expression of the 1.4-kb *UL24* transcript that is processed at the *UL24* poly(A) site. This hypothesis
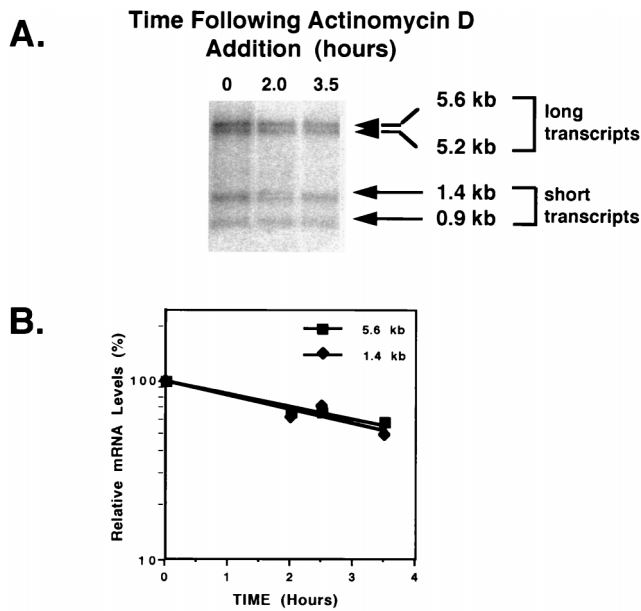
A.

FIG. 2. Stabilities of *UL24* transcripts. (A) Northern blot analysis of *UL24* 5.6-, 5.2-, 1.4-, and 0.9-kb transcripts isolated at various times after actinomycin D treatment. KOS1.1-infected Vero cells (10 PFU/cell) were treated with actinomycin D (5 μg/ml) at 5 h postinfection. At the times indicated, polyadenylated RNA was isolated from total RNA and analyzed by Northern blotting as described in Materials and Methods. Positions of the long (5.6- and 5.2-kb) and short (1.4- and 0.9-kb) transcripts are indicated by arrows. (B) Relative amounts of 1.4- and 5.6-kb *UL24* transcripts following actinomycin D treatment were determined by scanning the autoradiograms following Northern hybridization.

predicted that deletion of the *ICP27* gene would decrease levels of the 1.4-kb mRNA but not the larger 5.6-kb transcript.

Therefore, we infected Vero cells with three *ICP27* mutants, *d*27, *n*263R, and *n*504R (48), and determined the relative levels of the 1.4- and 5.6-kb *UL24* transcripts. Mutant *d*27 harbors a 1.6-kbp deletion at the *ICP27* locus and is devoid of any known *ICP27* function. Mutant *n*263R contains a nonsense codon at *ICP27* codon 264 and expresses only the N-terminal half of the protein; mutant *n*504R has a nonsense codon at *ICP27* codon 505 and lacks eight codons from the ICP27 C terminus. Additionally, both *d*27 and *n*263R accumulate reduced levels of viral DNA (6 to 23% of wt levels), whereas *n*504R synthesizes viral DNA levels similar to those of wt virus. The three *ICP27* mutants do not express true late (γ-2) genes; *n*504R expresses wt levels of leaky-late (γ-1) genes, mutant *n*263R is able to express some γ-1 genes, and mutant *d*27 does not fully activate the expression of most γ-1 genes (48). All three viral mutants are unable to form plaques on Vero cells but can replicate on the complementing cell line V27 (48).

RNA was isolated from Vero cells infected with wt, *d*27, *n*263R, or *n*504R at 5 and 10 h postinfection and analyzed by Northern blot hybridization (Fig. 3). The data were quantified by scanning the resulting autoradiograms. At 5 h h-postinfection, the levels of both short and long *UL24* transcripts were lower in mutant-infected cells than in wt-infected cells. However, by 10 h postinfection in the mutant-infected cells, the levels of the short transcripts were similar to or exceeded those in the wt-infected cells at any time postinfection (Fig. 3). In contrast, at 10 h postinfection, the levels of long *UL24* transcripts were reduced in *n*504R-infected cells and were even more dramatically reduced in cells infected with *d*27 or *n*263R compared to wt-infected cells (Fig. 3). Accordingly, at 10 h postinfection, the molar ratios of the short to long transcripts in *n*504R-, *d*27-, and *n*263R-infected cells were 0.7, 9.2, and

>10.0, respectively. The molar ratios for *d*27 and *n*263R were much higher than that of the wt virus (0.1).

Previous results have shown that *ICP27* is required for γ-2 gene expression and can stimulate the expression of certain γ-1 genes (32, 48, 49, 51, 52). The 5.6-kb transcript is expressed with γ-1 kinetics (6). To determine if the substantially decreased 5.6-kb transcript levels in the three *ICP27* mutants resulted solely from a general inability of the mutants to express γ-1 genes, the Northern blot in Fig. 3 was stripped and reprobed for the HSV γ-1 gene *gB*. At 10 h postinfection, there was somewhat less *gB* mRNA in cells infected with the three ICP27 mutants than in wt-infected cells (Fig. 3, middle). Next, we reprobed the blot in Fig. 3 for *ICP8*, an HSV β gene. Consistent with previous results (32, 64), ICP8 expression was increased or unaffected in *ICP27* mutant-infected cells. In particular, there was ample expression of *gB* and *ICP8* transcripts at 5 h postinfection; thus, the low levels of *UL24* transcripts detected at this time were not due to trivial causes such as poor RNA recovery. Taken together, these results indicate that in *ICP27* mutant-infected cells, the reduced expression of the long *UL24* transcripts at 10 h postinfection was a bona fide property of the ICP27 mutants.

The *ICP27*-complementing cell line V27 restores the expression of the 5.6-kb transcript by *d*27. To determine if the observed decrease in 5.6-kb transcripts relative to 1.4-kb transcripts in the *d*27-infected cells was due to absence of ICP27, we asked whether the wt ratio of 1.4-kb transcript to 5.6-kb transcript could be restored by *d*27 infection of V27 cells, an *ICP27*-complementing cell line (48). Vero or V27 cells were infected with wt virus or mutant *d*27, and total RNA was isolated, poly(A) selected, and analyzed by Northern blot hybridization. At 10 h postinfection, the levels of the 1.4-kb transcript were similar in all cases; however, the levels of the 5.6-kb transcript were drastically reduced in *d*27-infected Vero cells. In this experiment, *d*27 infection of Vero cells yielded a ratio of 1.4-kb transcripts to 5.6-kb transcript of 1.9 (Fig. 4, lane 3). However, when the *ICP27*-complementing cell line V27 was infected with *d*27, the ratio of 1.4-kb transcript to 5.6-kb transcript was similar to that of wt-infected V27 cells (Fig. 4, lanes 2 and 4). Similar ratios were observed in wt- and *d*27-infected V27 cells at 7.5 h postinfection (16). Thus, the reduced levels of 5.6-kb *UL24* transcripts observed in *d*27-infected Vero cells can be attributed to the absence of ICP27.

The reduced levels of the 5.6-kb *UL24* transcript are not due solely to inhibition of DNA replication. The results in Fig. 3 showed that cells infected with all *ICP27* mutants accumulated reduced amounts of *UL24* transcripts compared to wt-infected
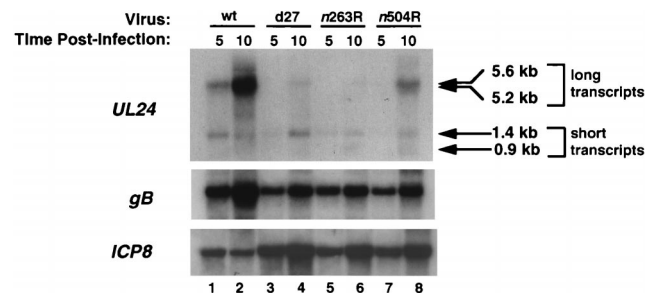

FIG. 3. Northern blot hybridization of total RNA isolated from cells infected with wt virus or *UL24* mutant viruses *d*27, *n*263R, and *n*504R at 5 or 10 h postinfection. The blot was hybridized with probes specific for *UL24* (top), *gB* (middle), and *ICP8* (bottom) as described in Materials and Methods. Positions of the long (5.6- and 5.2-kb) and short (0.9- and 1.4-kb) transcripts are indicated by arrows.
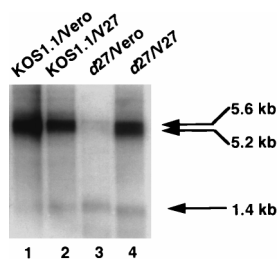
FIG. 4. Northern blot analysis of *UL24* 5.6- and 1.4-kb transcripts isolated from Vero or V27 cells infected with wt virus or the *ICP27* deletion mutant virus *d*27. Cells were infected at 10 PFU/ml, and polyadenylated mRNA was isolated from total RNA isolated at 10 h postinfection as described in Materials and Methods. Position of the 5.6-, 5.2-, and 1.4-kb transcripts are indicated by arrows.

cells. Only when cells were infected with mutant *n*504R were levels of the 5.6-kb transcript readily detectable at 10 h postinfection (Fig. 3, lane 8). As mutant *n*504R replicates viral DNA efficiently (48), it was conceivable that the low levels of the 5.6-kb transcript in *d*27- and *n*263R-infected cells resulted solely from reduced DNA replication and not the absence of *ICP27*. To determine if inhibition of viral DNA synthesis was responsible for the low 5.6-kb transcript levels, we infected Vero cells with either wt or *d*27 in the presence of the viral DNA synthesis inhibitor PAA. Northern blot hybridization of poly(A)-selected RNA isolated at 5.5 h postinfection (Fig. 5) showed that in the presence of PAA, the 5.6-kb transcript was evident in wt-infected cells, with a 1.4-kb/5.6-kb transcript ratio of 0.2, whereas in *d*27-infected cells the 5.6-kb transcript was not detected and the 1.4-kb/5.6-kb transcript ratio was >10.0. Similar amounts of *gB* transcripts were observed for the two viruses when the blot shown in Fig. 5 was stripped and reprobed with a *gB*-specific probe (16). Thus, the decrease in the 5.6-kb transcript in *d*27-infected cells was not due solely to reduced DNA synthesis.

DISCUSSION

In this study, we have investigated further the temporal regulation of two *UL24* transcripts that are transcribed from the same promoter. Our data argue that during viral infection, the temporal expression of the 1.4- and 5.6-kb *UL24* transcripts is not due to altered mRNA stabilities. This finding supports the hypothesis that the temporal regulation of the 1.4- and 5.6-kb *UL24* transcripts is governed, at least in part, at the level of differential polyadenylation. The promoter-proximal *UL24* poly(A) site, which generates the 1.4-kb transcript, should be more efficiently used than the downstream *UL26* poly(A) site because it is encountered first by the processing machinery (11, 66). Instead, except at very early times postinfection, 5.6-kb transcripts processed at the *UL26* poly(A) site are more abundant than 1.4-kb transcripts processed at the *UL24* poly(A) site; this finding indicates that during viral infection, the *UL24* site is operationally weak. Using a series of *ICP27* mutants, we found that ICP27, which has been suggested to stimulate usage of weak polyadenylation sites, was not required for 3′ processing of the 1.4-kb *UL24* transcript. We did find, however, that the accumulation of the 5.6-kb transcript required ICP27. Taken together, our results suggest that ICP27 does not stimulate usage of the operationally weak *UL24* poly(A) site but may instead play a role in increasing 5.6-kb transcript levels.

ICP27 does not stimulate 3′ processing at the *UL24* poly(A) site. Our results showing that ICP27 is not required for utilization of the operationally weak *UL24* polyadenylation site appear to differ from results of transient transfection and cell-free polyadenylation assays (35, 57) which suggested that ICP27 stimulates the expression of genes containing nonoptimal or inefficiently utilized polyadenylation signals (35, 57). One interpretation of this apparent difference is that transient transfection and in vitro assays may not accurately reflect the environment within HSV-infected cells. For example, during adenovirus infection, a switch from early to late polyadenylation site usage requires a *cis* change in the viral genome (11); thus, viral DNA alterations in *cis* may be needed for bona fide regulation by ICP27. It is also possible that the *UL24* poly(A) site is not representative of other inefficiently utilized polyadenylation sites in that it does not require ICP27 for 3′ processing.

It is thought that cleavage-stimulatory factor is the rate-limiting factor in determining polyadenylation site usage (13, 14, 63). This multisubunit protein binds GU- or U-rich tracts 3′ to the poly(A) site signal, AAUAAA (30, 66). Both the *UL24* and *UL26* poly(A) sites contain a GU-rich element downstream of the hexanucleotide AAUAAA but not other previously identified consensus elements (31, 53). In vitro polyadenylation assays have shown that four U's are sufficient for binding of the cleavage-stimulatory factor 64-kDa subunit and that the distance of this site from the AAUAAA motif can influence the polyadenylation site strength (30). This is of interest since in the *UL24* 3′ untranslated region (UTR) the U_4 element is 46 nucleotides downstream of the AAUAAA whereas in the *UL26* 3′ UTR the distance between the hexanucleotide AAUAAA and the U tract is shorter, with U_7 35 nucleotides downstream. Whether this is responsible for the weak utilization of the *UL24* poly(A) site remains to be determined.

Possible mechanisms of ICP27 in modulating the 5.6-kb transcript accumulation. Our data show that ICP27 is required for efficient accumulation of the 5.6-kb transcript. Because the 1.4- and 5.6-kb transcripts are transcribed from the same promoter (6), it is apparent that ICP27 does not selectively stimulate transcriptional initiation of the 5.6-kb transcript over the 1.4-kb transcript. Rather, ICP27 must exert its selective effects on the 5.6-kb transcript at a step subsequent to transcriptional initiation. We can envision several mechanisms by which ICP27 may stimulate the expression of the *UL24* 5.6-kb transcript at a step subsequent to transcriptional initiation: by promoting transcriptional elongation and thereby extending the transcripts to the *UL26* poly(A) signal, by stimulating poly(A) site selection at the *UL26* site, by altering mRNA transport, by stabilizing the 5.6-kb transcript, or by a combination of two or more of these processes. (It is, of course, possible that ICP27 acts both at one of these steps and during transcriptional initiation and that these two processes are linked; however, the
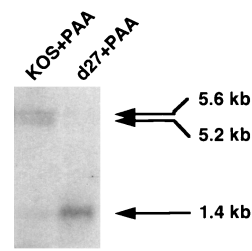

FIG. 5. Northern blot analysis of polyadenylated *UL24* 5.6- and 1.4-kb transcripts isolated from wt- or *d*27-infected Vero or V27 cells at 5.5 h postinfection in the presence of PAA (400 μg/ml). In this experiment, polyadenylated mRNA was selected from cytoplasmic RNA. Cells were infected with virus at 20 PFU/cell as described in Materials and Methods. Positions of the 5.6-, 5.2-, and 1.4-kb transcripts are indicated by arrows.

ensuing discussion will focus on each of these promoter-distal steps.)

Although there is no direct evidence that ICP27 can stimulate transcriptional elongation, this could explain why expression of the longer 5.6-kb *UL24* transcript requires ICP27. Evidence suggests that ICP27 may be required for an HSV-induced factor called LPF (36, 37). This factor has been shown to promote 3′ processing at promoter distal poly(A) sites at late times postinfection (37). Ordinarily, the efficiency of 3′ processing is reduced by increasing the distance between the 5′ end of the mRNA and the polyadenylation site; LPF abrogates this effect (37). If ICP27 is indeed a component of LPF, it may act by increasing transcriptional elongation, permitting efficient utilization of the *UL26* poly(A) site, located roughly 4,000 nucleotides downstream of the *UL24* poly(A) site. A role for ICP27 in increasing transcriptional elongation could also explain why Uprichard and Knipe found that of two differentially polyadenylated *UL52* mRNAs, accumulation of the larger transcript was especially dependent on ICP27 expression (64). It is also possible that ICP27 is ordinarily required for suppression of polyadenylation late in infection and that this permits the synthesis of longer transcripts.

The possibility that ICP27 promotes usage of the *UL26* poly(A) site is consistent with previous observations that ICP27 regulation of certain genes is 3′-end dependent (57) and that in vitro, ICP27 stimulates processing at certain late polyadenylation sites (35, 36). Recently, it has been shown that the carboxy-terminal domain of RNA polymerase II can specifically bind 3′-end processing factors (33). Thus, it is possible that ICP27 interacts simultaneously with the elongating polymerase II complex and 3′-end processing apparatus. Alternatively, direct binding of ICP27 to nascent transcripts could also promote use of the *UL26* poly(A) site.

ICP27 has been implicated in nuclear and cytoplasmic shuttling (38a, 42, 55a, 61). It has been proposed that ICP27 may stimulate late gene expression by specifically transporting late gene mRNAs to the cytoplasm (38a, 61). Thus, mRNA regulatory elements present within the 5.6-kb but not the 1.4-kb transcript may be required for export and expression of the 5.6-kb transcript at late times postinfection. It has also been proposed that ICP27 stabilizes mRNAs (2, 39, 60). In the three *ICP27* mutants tested, the peak levels of the 5.6-kb transcript, but not the 1.4-kb transcript, were specifically decreased compared to wt-infected cells. Thus, although the 1.4- and 5.6-kb transcripts exhibited similar stabilities in the presence of ICP27, we cannot rule out the possibility that the stability of the 5.6-kb transcript is decreased in the absence of functional ICP27. The extremely low amounts of long transcripts present in *d*27- and *n*263R-infected cells make it difficult to address this issue directly. It has been suggested that the ability of ICP27 to stabilize labile mRNAs resides in its extreme C terminus (2). This observation is interesting in light of the lower levels of long transcript observed in *n*504R-infected cells relative to wt-infected cells, since the ICP27 synthesized by *n*504R lacks the C-terminal eight amino acids.

Studies of ICP27 have been complicated by the multifunctional nature of the protein. The effect of *ICP27* mutants on *UL24* transcript levels should prove useful in deciphering the role of ICP27. Since distinct *UL24* transcripts are generated from the same promoter but utilize different polyadenylation sites, the apparent effects of ICP27 on transcriptional initiation (54) can be separated from regulation occurring subsequently. Thus, the *UL24* gene should provide a useful model for elucidating ICP27 function.

REFERENCES

1. **Block, T., and R. Jordan.** 1988. Herpes simplex virus type 1 alpha gene containing plasmids can inhibit expression regulated from an alpha promoter in CV-1 but not HeLa cells. Virus Res. **11:**269–279.
2. **Brown, C. R., M. S. Nakamura, J. D. Mosca, and G. S. Hayward.** 1995. Herpes simplex virus *trans*-regulatory protein ICP27 stabilizes and binds to 3′ ends of labile mRNA. J. Virol. **69:**7187–7195.
3. **Casey, J. L., and J. L. Gerin.** 1995. Hepatitis D virus RNA editing: specific modification of adenosine in the antigenomic RNA. J. Virol. **69:**7593–7600.
4. **Coen, D. M., H. E. Fleming, Jr., L. K. Leslie, and M. J. Retondo.** 1985. Sensitivity of arabinosyladenine-resistant mutants of herpes simplex virus to other antiviral drugs and mapping of drug sensitivity mutations to the DNA polymerase locus. J. Virol. **53:**477–488.
5. **Coen, D. M., S. P. Weinheimer, and S. L. McKnight.** 1986. A genetic approach to promoter recognition during trans induction of viral gene expression. Science **234:**53–59.
6. **Cook, W. J., and D. M. Coen.** 1996. Temporal regulation of herpes simplex virus type 1 *UL24* mRNA expression via differential polyadenylation. Virology **218:**204–213.
7. **Cook, W. J., B. Gu, N. A. DeLuca, E. B. Moynihan, and D. M. Coen.** 1995. Induction of transcription by a viral regulatory protein depends on the relative strengths of functional TATA boxes. Mol. Cell. Biol. **15:**4998–5006.
8. **Cullen, B. R., and W. C. Greene.** 1989. Regulatory pathways governing HIV-1 replication. Cell **58:**423–426.
9. **Denome, R. M., and C. N. Cole.** 1988. Patterns of polyadenylation site selection in gene constructs containing multiple polyadenylation signals. Mol. Cell. Biol. **8:**4829–4839.
10. **Everett, R. D.** 1986. The products of herpes simplex virus type 1 (HSV-1) immediate early genes 1, 2, and 3 can activate HSV-1 gene expression in *trans*. J. Gen. Virol. **67:**2507–2513.
11. **Falck-Pedersen, E., and J. Logan.** 1989. Regulation of poly(A) site selection in adenovirus. J. Virol. **63:**1–4.
12. **Fortes, P., A. Beloso, and J. Ortin.** 1994. Influenza virus NS1 protein inhibits pre-mRNA splicing and blocks mRNA nucleocytoplasmic transport. EMBO J. **13:**704–712.
13. **Gilmartin, G. M., and J. R. Nevins.** 1991. Molecular analysis of two poly(A) site-processing factors that determine recognition and efficiency of cleavage of the pre-mRNA. Mol. Cell. Biol. **11:**2432–2438.
14. **Gilmartin, G. M., and J. R. Nevins.** 1989. An ordered pathway of assembly of components required for polyadenylation site recognition and processing. Genes Dev. **3:**2180–2189.
15. **Hann, L. E., and L. Gehrke.** 1995. mRNAs containing the unstructured 5′ leader sequence of alfalfa mosaic virus RNA 4 translate inefficiently in lysates from poliovirus-infected HeLa cells. J. Virol. **69:**4986–4993.
16. **Hann, L. E., D. M. Knipe, and D. M. Coen.** Unpublished results.
17. **Hann, L. E., A. Webb, J. M. Cai, and L. Gehrke.** 1997. Identification of a competitive translation determinant in the 3′ untranslated region of alfalfa mosaic virus coat protein mRNA. Mol. Cell. Biol. **17:**2005–2013.
18. **Hardwicke, M. A., and R. M. Sandri-Goldin.** 1994. The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. J. Virol. **68:**4797–4810.
19. **Hardwicke, M. A., P. J. Vaughn, R. E. Sekulovich, R. E. O'Connor, and R. M. Sandri-Goldin.** 1989. The regions important for the activator and repressor functions of herpes simplex virus type 1 α protein ICP27 map to the C-terminal half of the molecule. J. Virol. **63:**4590–4602.
20. **Hardy, W. R., and R. M. Sandri-Goldin.** 1994. Herpes simplex virus inhibits host cell splicing and regulatory protein ICP27 is required for this effect. J. Virol. **68:**7790–7799.
21. **Hibbard, M. K., and R. M. Sandri-Goldin.** 1995. Arginine-rich regions succeeding the nuclear localization region of the herpes simplex virus type 1 regulatory protein ICP27 are required for efficient nuclear localization and late gene expression. J. Virol. **69:**4656–4667.
22. **Horikami, S., and S. Moyer.** 1991. Synthesis of leader RNA and editing of the P mRNA during transcription by purified measles virus. J. Virol. **65:**5342–5347.
23. **Hughes, R. G., and W. H. Munyon.** 1975. Temperature-sensitive mutants of herpes simplex virus type 1 defective in lysis but not in transformation. J. Virol. **16:**275–283.
24. **Kennedy, I. M., J. K. Haddow, and J. B. Clements.** 1991. A negative regulatory element in the human papillomavirus type 16 genome acts at the level of late mRNA stability. J. Virol. **65:**2093–2097.
25. **Kerry, J. A., M. A. Priddy, C. P. Kohler, T. L. Staley, D. Weber, T. R. Jones, and R. M. Stenberg.** 1997. Translational regulation of the human cytomegalovirus pp28 (UL99) late gene. J. Virol. **71:**981–987.
26. **Kibler, P. K., J. Duncan, B. D. Keith, T. Hupel, and J. R. Smiley.** 1991.

Regulation of herpes simplex virus true late gene expression: sequences downstream from the US11 TATA box inhibit expression from an unreplicated template. J. Virol. **65:**6749–6760.

27. **Lee, C. K., and D. M. Knipe.** 1983. Thermolabile in vivo DNA-binding activity associated with a protein encoded by mutants of herpes simplex virus type 1. J. Virol. **46:**909–911.

28. **Levin, J. K., and D. M. Knipe.** Unpublished data.

29. **Levitt, N., A. Briggs, A. Gil, and N. J. Proudfoot.** 1989. Definition of an efficient synthetic poly(A) site. Genes Dev. **3:**1019–1025.

30. **MacDonald, C. C., J. Wilusz, and T. Shenk.** 1994. The 64-kilodalton subunit of the CstF polyadenylation factor binds to pre-mRNAs downstream of the cleavage site and influences cleavage site location. Mol. Cell. Biol. **14:**6647–6654.

31. **MacLauchan, J., D. Gaffney, J. L. Whitton, and J. B. Clements.** 1985. The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3′ termini. Nucleic Acids Res. **13:**1347–1367.

32. **McCarthy, A. M., L. McMahan, and P. A. Schaffer.** 1989. Herpes simplex virus type 1 *ICP27* mutants exhibit altered patterns of transcription and are DNA deficient. J. Virol. **63:**18–27.

33. **McCracken, S., N. Fong, K. Yankulov, S. Ballantyne, G. Pan, J. Greenblatt, S. D. Patterson, M. Wickens, and D. Bentley.** 1997. The C-terminal domain of RNA polymerase II couples mRNA processing to transcription. Nature **385:**357–361.

34. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.

35. **McGregor, F., A. Phelan, J. Dunlop, and J. B. Clements.** 1996. Regulation of herpes simplex virus poly(A) site usage and the action of immediate-early protein IE63 in the early-late switch. J. Virol. **70:**1931–1940.

36. **McLauchlan, J., A. Phelan, C. Loney, R. M. Sandri-Goldin, and J. B. Clements.** 1992. Herpes simplex virus IE63 acts at the posttranscriptional level to stimulate virus mRNA 3′ processing. J. Virol. **66:**6939–6945.

37. **McLauchlan, J., S. Simpson, and J. B. Clements.** 1989. Herpes simplex virus induces a processing factor that stimulates poly(A) site usage. Cell **59:**1093–1105.

38. **McMahan, L., and P. A. Schaffer.** 1990. The repressing and enhancing functions of the herpes simplex virus regulatory protein ICP27 map to C-terminal regions and are required to modulate viral gene expression very early in infection. J. Virol. **1990:**3471–3485.

38a.**Mears, W. E., and S. A. Rice.** 1998. The herpes simplex virus immediate-early protein ICP27 shuttles between nucleus and cytoplasm. Virology **242:**128–137.

39. **Mosca, J. D., P. M. Pitha, and G. S. Hayward.** 1992. Herpes simplex virus infection selectively stimulates accumulation of beta interferon reporter gene mRNA by a posttranscriptional mechanism. J. Virol. **66:**3811–3822.

40. **Nevins, J. R., and M. C. Wilson.** 1981. Regulation of adenovirus-2 gene expression at the level of transcriptional termination and RNA processing. Nature **290:**113–118.

41. **Phelan, A., M. Carmo-Fonseca, J. McLauchlan, A. L. Lamond, and J. B. Clements.** 1993. A herpes simplex virus type 1 immediate-early gene product IE63, regulates small nuclear ribonucleoprotein distribution. Proc. Natl. Acad. Sci. USA **90:**9056–9060.

42. **Phelan, A., and J. B. Clements.** 1997. Herpes simplex virus type 1 immediate early protein IE63 shuttles between nuclear compartments and the cytoplasm. J. Gen. Virol. **78:**3327–3331.

43. **Phelan, A., J. Dunlop, and J. B. Clements.** 1996. Herpes simplex virus type 1 protein IE63 affects the nuclear export of virus intron-containing transcripts. J. Virol. **70:**5255–5265.

44. **Rafield, L. F., and D. M. Knipe.** 1984. Characterization of the major mRNA transcribed from genes for glycoprotein B and the DNA-binding protein ICP8 of herpes simplex type 1. J. Virol. **49:**960–969.

45. **Read, G. S., J. A. Sharp, and W. C. Summers.** 1984. In vitro and in vivo transcription initiation sites on the *tk*-encoding BamH1 Q fragment of HSV-1 DNA. Virology **138:**368–372.

46. **Read, G. S., and W. C. Summers.** 1982. In vitro transcription of the thymidine kinase gene of herpes simplex virus. Proc. Natl. Acad. Sci. USA **79:**5215–5219.

47. **Rice, S. A., and D. M. Knipe.** 1988. Gene-specific transactivation by herpes

48. **Rice, S. A., and D. M. Knipe.** 1990. Genetic evidence for two distinct trans-activation functions of the herpes simplex virus α protein ICP27. J. Virol. **64:**1704–1715.

49. **Rice, S. A., and V. Lam.** 1994. Amino acid substitution mutations in the herpes simplex virus ICP27 protein define an essential gene regulation function. J. Virol. **68:**823–833.

50. **Rice, S. A., L. S. Su, and D. M. Knipe.** 1989. Herpes simplex virus α protein ICP27 possesses separable positive and negative regulatory activities. J. Virol. **63:**3399–3407.

51. **Rice, S. A., V. Lam, and D. M. Knipe.** 1993. The acidic amino-terminal region of herpes simplex virus type 1 alpha protein ICP27 is required for an essential lytic function. J. Virol. **67:**1778–1787.

52. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55:**796–805.

53. **Sadofsky, M., and J. C. Alwine.** 1984. Sequences on the 3′ side of the hexanucleotide AAUAAA effect efficiency of cleavage at the polyadenylation site. Mol. Cell. Biol. **4:**1460–1468.

54. **Samaniego, L. A., A. L. Webb, and N. A. DeLuca.** 1995. Functional interactions between herpes simplex virus immediate-early proteins during infection: gene expression as a consequence of ICP27 and different domains of ICP4. J. Virol. **69:**5705–5715.

55. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

55a.**Sandri-Goldin, R. M.** 1998. ICP27 mediates HSV RNA export by shuttling through a leucine-rich nuclear export signal and binding viral intronless RNAs through an RGG motif. Genes Dev. **12:**868–879.

56. **Sandri-Goldin, R. M., M. K. Hibbard, and M. A. Hardwicke.** 1995. The C-terminal repressor region of herpes simplex virus type 1 ICP27 is required for the redistribution of small nuclear ribonucleoprotein particles and splicing factor SC35; however, these alterations are not sufficient to inhibit host cell splicing. J. Virol. **69:**6063–6076.

57. **Sandri-Goldin, R. M., and G. E. Mendoza.** 1992. A herpesvirus regulatory protein appears to act post-transcriptionally by affecting mRNA processing. Genes Dev. **6:**848–863.

58. **Schmechel, S., M. Chute, P. Skinner, R. Anderson, and L. Schiff.** 1997. Preferential translation of reovirus mRNA by a sigma 3-dependent mechanism. Virology **232:**62–73.

59. **Scott, J., and M. Imperiale.** 1997. Promoter-proximal poly(A) sites are processed efficiently, but the RNA products are unstable in the nucleus. Mol. Cell. Biol. **17:**2127–2135.

60. **Smith, I. L., M. A. Hardwicke, and R. M. Sandri-Goldin.** 1992. Evidence that the herpes simplex virus immediate early protein ICP27 acts post-transcriptionally during infection to regulate gene expression. Virology **186:**74–86.

61. **Soliman, T. M., R. M. Sandri-Goldin, and S. J. Silverstein.** 1997. Shuttling of the herpes simplex virus type 1 regulatory protein ICP27 between the nucleus and cytoplasm mediates the expression of late proteins. J. Virol. **71:**9188–9197.

62. **Su, L., and D. M. Knipe.** 1989. Herpes simplex virus alpha protein ICP27 can inhibit or augment viral gene transactivation. Virology **170:**496–504.

63. **Takagaki, Y. J., C. C. Manley, C. C. MacDonald, J. Wilusz, and T. Shenk.** 1990. A multisubunit factor, CstF, is required for polyadenylation of mammalian pre-mRNAs. Genes Dev. **4:**2112–2120.

64. **Uprichard, S. L., and D. M. Knipe.** 1996. Herpes simplex ICP27 mutant viruses exhibit reduced expression of specific DNA replication genes. J. Virol. **70:**1969–1980.

65. **Volchkov, V., V. Becker, V. Volchkova, V. Ternovoj, A. Kotov, S. Netesov, and H. Klenk.** 1995. GP mRNA of Ebola virus is edited by the Ebola virus polymerase and T7 and vaccinia virus polymerase. Virology **214:**421–430.

66. **Weiss, E. A., G. M. Gilmartin, and J. R. Nevins.** 1991. Poly(A) site efficiency reflects the stability of complex formation involving the downstream element. EMBO J. **10:**215–219.

67. **Wilkie, N. M., R. P. Eglin, P. G. Sanders, and J. B. Clements.** 1980. The association of herpes simplex virus with squamous carcinoma cells of the cervix, and studies of the virus thymidine kinase gene. Proc. R. Soc. Lond. Biol. Sci. **210:**411–421.

68. **Yager, D., and D. M. Coen.** 1990. Translational regulation of herpes simplex virus DNA polymerase. J. Virol. **64:**2217–2225.