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Mutations in the Major DNA-Binding Protein Gene of Herpes Simplex Virus Type 1 Result in Increased Levels of Viral Gene Expression

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We have examined the effect of temperature-sensitive mutations in the herpes simplex virus 1 DNA-binding protein gene on viral gene expression. We have found that at the nonpermissive temperature, the synthesis of certain immediate early, early, and late viral polypeptides was greater in cells infected with the temperature-sensitive mutants than in cells infected with the wild-type virus. This effect was independent of the requirement for this viral protein for viral DNA replication. The altered rate of synthesis of viral proteins was due to a thermolabile gene product. Cells infected with these mutants at the permissive temperature and then shifted to the nonpermissive temperature exhibited enhanced levels of viral gene expression. The addition of actinomycin D at the time of the temperature shift prevented the alteration in viral protein synthesis. Therefore, continuing transcription is required for this change in gene expression. Northern blot analysis of cytoplasmic RNA showed that the steady-state level of specific viral transcripts expressed from parental virus genomes was greater in cells infected by these mutants at the nonpermissive temperature. These results indicate that the major DNA-binding protein of herpes simplex virus type 1 acts as a negative regulator of viral gene expression by affecting the abundance of cytoplasmic viral mRNAs.

The regulation of herpes simplex virus (HSV) gene expression is a complex process dependent on both cellular and viral factors. Upon infection of the host cell, a limited portion of the viral genome is transcribed by the host cell RNA polymerase II to yield immediate early mRNAs. These are translated to yield the immediate early proteins. These proteins alter transcription or processing of viral mRNA such that a new class of mRNAs is produced. The proteins encoded by these mRNAs, the early proteins, are required for viral DNA replication. After viral DNA replication, the synthesis of certain viral proteins is initiated, whereas the synthesis of other proteins is enhanced. These are collectively termed the late viral proteins (29).

The immediate early proteins are synthesized at maximal rates within a few hours after infection of the host cell. The synthesis of the immediate early proteins is reduced concomitantly with the increase in synthesis of early proteins. The investigation of the kinetics and the requirements for synthesis of viral polypeptides led Honess and Roizman (13, 14) to propose that early gene products negatively regulate immediate early gene expression. The specific early gene products involved in this process were not identified. By analysis of immediate early temperature-sensitive mutants, Watson and Clements (34) and Dixon and Schaffer (9) showed that inactivation of the infected cell polypeptide 4 (ICP4) gene function led to increased synthesis of immediate early RNA and proteins, respectively. Thus, both immediate early and early gene products may negatively regulate immediate early gene expression.

We have been studying the maturation and function of ICP8, the major HSV DNA-binding protein. This early protein accumulates in the cell nucleus where it binds to viral DNA (19, 20). ICP8 is an essential function for viral DNA replication (6). ICP8 binds more tightly to single-stranded DNA than to double-stranded DNA in vitro (18, 25).

The complementation group 1-1 is likely to contain mutants defective in ICP8. Mutants in this group have lesions that map in or near the ICP8 coding sequences (6, 37). These mutants encode major DNA-binding proteins that exhibit altered antigenic properties (37) or DNA-binding

activities at the nonpermissive temperature (20). In this report, we examined the effect of these defects in ICP8 on viral gene expression.

MATERIALS AND METHODS

Cells, viruses, and inhibitors. The procedures used for propagating and titrating viral stocks have been described (19). Stocks of temperature-sensitive viruses and wild-type viruses were titrated in parallel at 33°C. The origins of HSV type 1 (HSV-1) mP and mP*ts*HA1 have been described (6, 12). HSV-1 strains KOS1.1 and KOS1.1*ts*13 (reference 15; L. Holland, R. Sandri-Goldin, J. C. Glorioso, and M. Levine, manuscript in preparation) were provided by R. Sandri-Goldin and M. Levine, University of Michigan, Ann Arbor. Strains KOS and KOS*ts*C7 (27, 28) were provided by P. Schaffer, Dana-Farber Cancer Institute, Boston, Mass. Sodium phosphonoacetate (PAA) was a gift of Abbott Labs, N. Chicago, Ill. PAA was used at 400 μg/ml, a concentration which totally inhibits viral DNA replication in Vero cells (M. Quinlan and D. Knipe, unpublished data). PAA was added at the time of infection and maintained throughout the incubation and labeling period. Actinomycin D was obtained from Calbiochem, La Jolla, Calif.

Labeling of infected cells. The infection of Vero cell monolayers at a multiplicity of 20 PFU per cell and the labeling of these cultures with [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) were performed in constant-temperature water baths as described previously (19). The 15-min labeling period was terminated by submerging the flasks in an ice bath. The cells were washed 3 times with phosphate-buffered saline at 0 to 4°C, scraped with a rubber policeman, and collected by centrifugation. The cell pellet was suspended in electrophoresis sample buffer (19) containing 1 mM phenylmethylsulfonyl fluoride. Proteins from these samples were analyzed by electrophoresis in 9.25% diallyltartardiamide cross-linked polyacrylamide gels as described previously (19). Portions of each sample containing equal quantities of cell lysate were loaded onto the gels. The autoradiograms shown and those used for tracing were exposures within the linear response range for the film. The labeling of cultures for 5 to 30 min also showed linear incorporation of label into viral polypeptide bands (data not shown). Autoradiograms were scanned with a Joyce-Loebl microdensitometer, and peak areas were determined with a Keufel and Esser planimeter.

RNA isolation and characterization. Vero cell monolayers were infected with the indicated HSV-1 strains and maintained in culture medium containing 400 μg of sodium PAA per ml. At 6 h postinfection, the cells were harvested, and cytoplasmic RNA was isolated by a modification of the method of Berk et al. (4). The cells were lysed with 1% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.) in 0.15 M NaCl–0.02 M Tris-hydrochloride (pH 7.9). Nuclei were separated from the cytosol fraction by centrifugation at 2,000 × *g* for 10 min at 4°C. The cytosol fraction was poured into an equal volume of a solution of 7 M urea, 0.35 M NaCl, 10 mM Tris-hydrochloride (pH 7.4), 10 mM EDTA, and 4% SDS. This was extracted twice with an equal volume of phenol (saturated with 0.15 M NaCl, 10 mM Tris-hydrochloride (pH 7.6), and 0.5 mM EDTA) and one-half volume of chloroform-isoamyl alcohol (24:1). Nucleic acids were precipitated by the addition of sodium acetate to 0.2 M and 2 volumes of ethanol followed by incubation at −20°C for 12 h. Samples of each RNA preparation (10 or 20 μg) were denatured and subjected to electrophoresis in 0.8% neutral agarose gels containing 2.2 M formaldehyde as described previously (16), except that ethidium bromide was not included in the gel. The RNA was transferred to nitrocellulose (Schleicher & Schuell Co., Keene, N.H.) in 10× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate [pH 7.0]) (31). The filters were preincubated in 50% formamide (Fluka, Hauppauge, N.Y.)–5 × SSC–0.02% Denhardt solution (8)–50 mM potassium phosphate buffer (pH 6.5)–250 μg of yeast RNA per ml–0.25% sodium dodecyl sulfate for 17 h at 42°C. Hybridizations were performed for 48 h with ^{32}P-labeled DNA probes (5 × 10^6 cpm per nitrocellulose strip) under these same conditions. The filters were washed twice in 2× SSC–0.1% sodium dodecyl sulfate at room temperature for 30 min and once in 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C for 1 h in a circulating water bath. Autoradiography was performed with Kodak XRP-5 film at room temperature or at −70°C with intensifying screens. Plasmid pBamHI-A′ (7) was a gift of R. Costa and E. Wagner, University of California, Irvine. This plasmid contains the HSV-1 *Bam*HI A′ DNA fragment (map coordinates 0.255 to 0.266) inserted in the *Bam*HI site of pBR322. Plasmid pSG18SalA (20) contains an HSV-1 *Sal*I DNA fragment (map coordinates 0.385 to 0.419, equivalent to the *Sal*I O fragment of HSV-1 strain F DNA [21]) inserted in the *Sal*I site of pBR325. Plasmid pSG-1 (10) was generously provided by M. Levine, University of Michigan, Ann Arbor. This plasmid contains the HSV-1 *Eco*RI JK DNA fragment inserted in the *Eco*RI site of pBR325. The *Bam*HI SP fragment is contained within the *Eco*RI JK fragment and arises from the junction of the L and S components of viral DNA (21).

Plasmid DNAs were isolated by CsCl equilibrium density gradient centrifugation of lysates from plasmid-containing cells (5). The HSV-1 DNA inserts in these plasmids were released from the vector DNA by digestion with the appropriate restriction enzyme. Restriction endonuclease digestions were performed under conditions specified by the supplier (New England Biolabs, Beverly, Mass.). The DNA fragments containing the HSV-1-specific sequences were isolated by electroelution from neutral agarose gels (23). These were labeled by nick translation (26) with [^{32}P]dCTP (800 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) to a specific activity of 5 × 10^7 to 7 × 10^7 cpm/μg.

RESULTS

Synthesis of viral proteins in cells infected with a DNA-binding protein mutant. The major DNA-binding protein, ICP8, encoded by the group 1-1 mutant *ts*13 is thermolabile for binding to viral DNA (20). Others have shown that group 1-1 mutants are defective for viral DNA replication (6, 37; L. Holland et al., manuscript in preparation). Viral DNA replication enhances late gene expression, and late gene products inhibit early

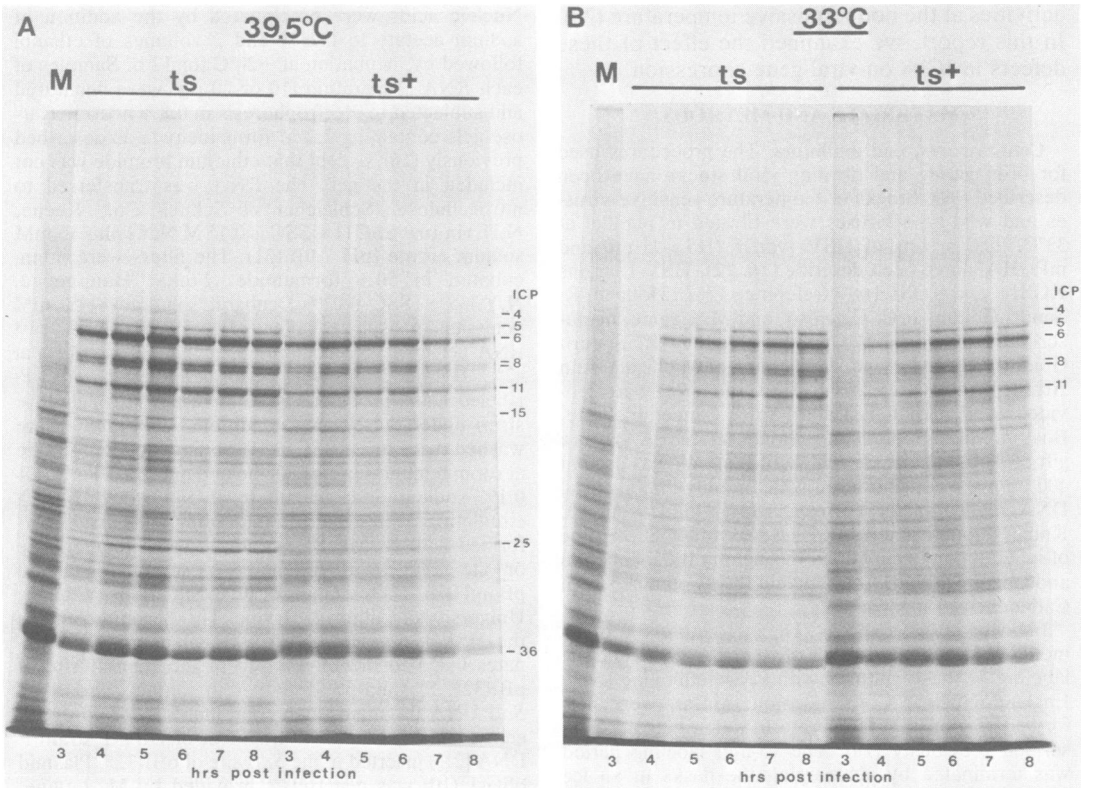

FIG. 1. Comparison of the rates of synthesis of viral proteins in cells infected with HSV-1 *ts*13 or ts^+ virus in the presence of PAA. Cells were infected with *ts*13 (ts) or KOS1.1 (ts^+) virus or mock-infected (M) in the presence of PAA. At the times indicated, the cells were labeled for 15 min with 6 μCi of [^{35}S]methionine per ml. Extracts were prepared, and the viral proteins were analyzed by gel electrophoresis as described in the text. An autoradiogram of the gel is shown. Some viral polypeptides (ICP) are indicated. The two forms of ICP8, differing in their degree of intramolecular disulfide bonding (17), are also indicated.

gene expression (29). The purpose of this study was to determine whether ICP8 affects viral gene expression independently of the requirement of this protein for viral DNA replication.

We compared the amounts of viral proteins synthesized during pulse-labeling periods in cells infected with *ts*13 or its wild-type parent, strain KOS1.1. To control for any effect that DNA replication might have on viral gene expression, the infected cultures were incubated with PAA, an inhibitor of the viral DNA polymerase. Replicate cultures were infected with KOS1.1 ts^+ or *ts*13 viruses and maintained in medium containing PAA at 33° or 39.5°C. At 1-h intervals between 3 to 8 h postinfection, the cells were pulse labeled with [^{35}S]methionine, and the labeled viral polypeptides were analyzed by electrophoresis and autoradiography. At 39.5°C, cells infected with *ts*13 synthesized increased amounts of certain viral proteins relative to the cells infected with the ts^+ virus (Fig. 1A). These included the early ICPs 6, 8, and 36 and the late ICPs 5, 11, 15, and 25. In addition, the immediate early ICP4 was synthesized in greater amounts and at later times in cells infected with *ts*13. At the permissive temperature (33°C), the amounts of viral proteins synthesized in cells infected with *ts*13 and the ts^+ virus were similar (Fig. 1B). Thus, under conditions in which viral DNA replication was uniformly inhibited in all cultures, the effect of the temperature-sensitive mutation was to increase the level of expression of several viral genes.

From this experiment, we quantitated the magnitude of the difference in the peak rates of synthesis of viral polypeptides in the mutant and wild-type infected cells. By microdensitometry and planimetry of the autoradiograms (Fig. 1), we determined the amount of labeled ICP8. At 39.5°C, the amount of synthesis of ICP8 in cells infected with *ts*13 was greater at all times than that observed in cells infected with ts^+ virus. The peak rate of synthesis of ICP8 (5 h postinfection) was approximately threefold greater in the mutant-infected cells (Fig. 2A). At 33°C, the amount of ICP8 synthesized in cells infected with the mutant and ts^+ virus was similar (Fig. 2B).

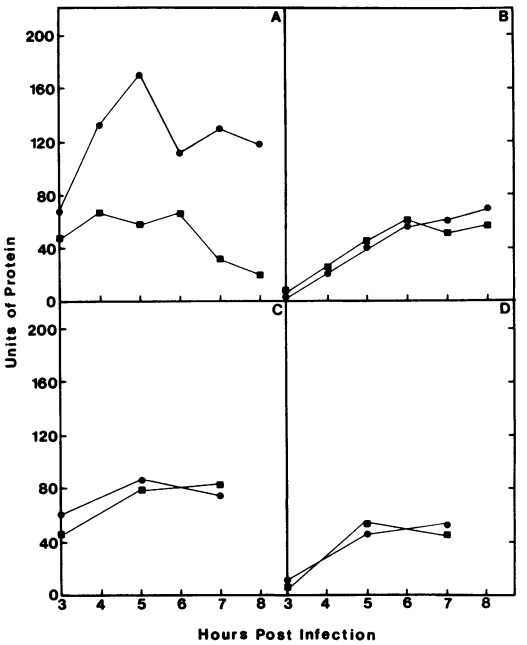

FIG. 2. Rates of synthesis of ICP8 in cells infected with DNA-negative mutants or ts^+ viruses. Infected cultures were labeled for 15 min at the times indicated, and viral proteins were analyzed as in Fig. 1. The intensity of the ICP8 bands was determined by microdensitometry and planimetry. (A and B) Symbols: ●, cells infected with *ts*13; ■, cells infected with ts^+ virus. (C and D) Symbols: ●, cells infected with *ts*C7; ■, cells infected with KOS ts^+. Cells were incubated at 39.5°C (A and C) or 33°C (B and D).

Comparison of late gene expression by ICP8 mutants and replicating wild-type virus. The expression of HSV late genes is inhibited when viral DNA replication is blocked (29). The results presented above demonstrate that the inhibition of late gene expression from parental DNA molecules was at least partially overcome by mutations in the major DNA-binding protein. It was of interest to compare the amount of late gene expression by ICP8 mutants with that of a replicating wild-type virus. Replicate cultures were infected at 39.5°C with the ICP8 mutant *ts*HA1 (6, 20) or its parent strain mP in the presence or absence of PAA. At 6 h postinfection, viral polypeptide synthesis was analyzed.

In cultures incubated with PAA, the amount of synthesis of early polypeptides (e.g., ICPs 6, 8, and 36), late polypeptides (e.g., ICPs 5, 11, 15, and 25) and the immediate early ICP4 was greater in cells infected with *ts*HA1 (Fig. 3, lanes 2 and 4). Thus, this ICP8 mutant also overproduced viral polypeptides at 39.5°C. In cultures incubated without PAA, synthesis of early proteins and of the immediate early ICP4 was greater in cells infected with *ts*HA1 than in cells infected with the ts^+ virus. However, synthesis of the late ICPs 5, 15, and 25 was greatest in cells infected with the ts^+ virus (Fig. 3). Thus, although the level of late gene expression by ICP8 mutants was greater than that observed with a nonreplicating wild-type virus, it was not as great as that observed in cells in which viral DNA replication was occuring. These results extend earlier studies by Conley et al. (6) showing that ICP8 mutants are defective in the accumulation of late gene products.

Characterization of other mutants defective for DNA replication. To determine whether an increased level of viral protein synthesis was a general characteristic of all mutants defective for DNA replication, we examined the kinetics

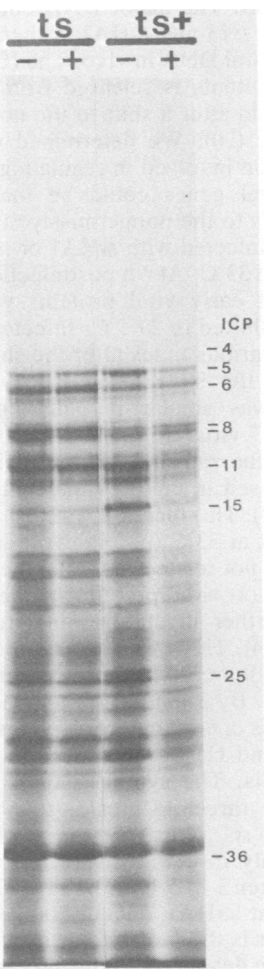

FIG. 3. Synthesis of viral proteins in cells infected with HSV-1 *ts*HA1 or ts^+ virus in the presence or absence of PAA. At 6 h postinfection at 39.5°C, the cells were labeled for 15 min with [^{35}S]methionine, and viral proteins were analyzed as in Fig. 1. The samples shown were from infection with *ts*HA1 (ts) or mP (ts^+) with (+) or without (−) PAA.

of protein synthesis in cells infected with the HSV-1 mutant KOS*ts*C7. This mutant has been shown to encode a thermolabile DNA polymerase, and it fails to replicate its DNA at the nonpermissive temperature (2). As a member of complementation group 1-3, it complements the ICP8 mutant *ts*HA1 (36). At 39.5 or 33°C, the amount of synthesis of ICP8 directed by the mutant or the wild-type virus was similar (Fig. 2C and D). The rates of synthesis of other viral proteins in cells infected with *ts*C7 and ts^+ virus were also similar (data not shown). These results indicate that a mutational block in DNA replication alone is not sufficient to account for the increased rates of viral protein synthesis described above.

Changes in viral protein synthesis after a temperature shift. The major DNA-binding protein encoded by *ts*13 and *ts*HA1 is thermolabile for binding to viral DNA in vivo. The ICP8 encoded by these mutants is released from viral DNA within 30 min after a shift to the nonpermissive temperature (20). We determined whether the gene function involved in regulating the expression of viral genes could be inactivated by shifting cells to the nonpermissive temperature. Cells were infected with *ts*HA1 or ts^+ virus and incubated at 33°C. At 4 h postinfection, after the initiation of early viral protein synthesis, the cells were shifted to 39.5°C. Infected cells were labeled at various times after the shift. Immediately after the shift to 39.5°C, viral protein synthesis was similar in cells infected with *ts*HA1 or ts^+ virus (Fig. 4). At 1 h after the shift to 39.5°C, the rates of viral protein synthesis were increased in cultures infected with either virus (Fig. 4). This increase was also observed in cultures left at 33°C (not shown), and thus this change was not related to the temperature shift. The levels of viral protein synthesis did not increase further in cultures infected with ts^+ virus (Fig. 4). However, the synthesis of viral proteins did increase further in cultures infected with *ts*HA1. By 3 h after the temperature shift, the synthesis of certain viral proteins (e.g., ICPs 4, 5, 6, 8, and 11) was enhanced in the mutant-infected cells. The synthesis of ICP8 was approximately threefold greater than in cells infected with ts^+ virus. Synthesis of ICP4 was detected only in cultures infected with *ts*HA1 and only after 3 to 5 h at 39.5°C. These results indicate that *ts*HA1 encodes a gene product which either is thermolabile or has a very short half-life. The deficiency of this product results in increased rates of viral protein synthesis. Similar results were obtained in temperature shift experiments involving *ts*13 (data not shown).

To further examine the mechanism by which ICP8 affects viral gene expression, we determined whether the alteration in viral protein synthesis after a temperature shift could be blocked by inhibiting transcription. In this experiment, cells were infected with *ts*13 or ts^+ virus and incubated at 33°C for 5 h. Actinomycin D was added to one half of the cultures. All cultures were then shifted to 39.5°C. The rates of synthesis of ICP8 were determined at this time or after a 4-h incubation period at this temperature. Cells infected with ts^+ virus synthesized similar amounts of ICP8 immediately after and 4 h after the shift to 39.5°C (Table 1). Cells infected with *ts*13 showed a threefold increase in the rate of synthesis of ICP8 after a 4-h incubation

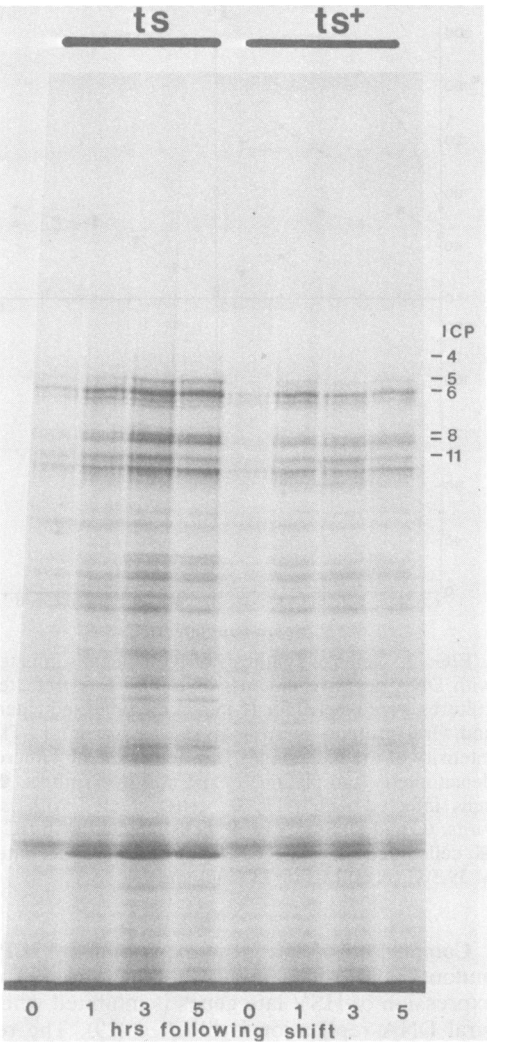

FIG. 4. Synthesis of viral proteins in infected cells after a temperature shift. Cells were infected with *ts*HA1 (ts) or mP (ts^+) at 33°C in the presence of PAA. At 4 h postinfection, the cultures were shifted to 39.5°C. At the times indicated after the shift, the cells were labeled with [^{35}S]methionine, and the labeled proteins were analyzed as in Fig. 1.

TABLE 1. Effect of actinomycin D on the overproduction of ICP8 after a temperature shift[a]

Virus	Actinomycin D	Amt of ICP8[b]	
		0 h	4 h
*ts*13	−	80	263
ts^+	−	99	119
*ts*13	+	76	53
ts^+	+	64	41

[a] Cells were infected at 33°C for 5 h. At this time, actinomycin D was added to one half of the cultures to a concentration of 10 μg/ml. All cultures were then shifted to 39°C for either 0 or 4 h and labeled for 15 min with [^{35}S]methionine at 39°C.

[b] The amount of ICP8 in arbitrary units determined by planimetry. These values represent the average of triplicate cultures.

period at 39.5°C. However, the addition of actinomycin D to cultures infected with ts^+ or *ts*13 virus caused the synthesis of ICP8 to be reduced to 64 and 70%, respectively, of the initial rate by 4 h after the temperature shift. Thus, continuing transcription is needed for the enhanced rates of ICP8 synthesis in cells infected with *ts*13 at 39.5°C. These results also indicate that the functional half-life of the ICP8 mRNA is similar in cells infected with *ts*13 and ts^+ viruses.

Accumulation of viral mRNA. The observation that the inhibition of transcription prevented the increased rates of protein synthesis in mutant-infected cells suggested that the ICP8 function may affect the level of viral mRNA accumulation. We assayed the level of viral-specific cytoplasmic RNA from cells infected with the mutant or ts^+ virus by Northern blot hybridization. As hybridization probes, we used cloned HSV-1 DNA fragments derived from regions of the genome that encode ICPs 4, 5, or 8. The *Bam*HI SP DNA fragment hybridizes with a 4.7- kilobase (kb) transcript that encodes ICP4 (1, 22, 35). This probe also contains sequences homologous to the 3.0-kb transcript encoding the immediate early ICP0 (1, 22). The *Bam*HI A′ DNA fragment hybridizes to a major transcript that encodes ICP5 and to several minor transcripts (reference 7; E. Wagner, personal communication). The pSG18SalA DNA fragment hybridizes to a major transcript of 4.5 kb. In vitro translation of mRNA that hybridizes to the pSG18SalA DNA fragment yields a polypeptide with a molecular weight of approximately 125,000. This polypeptide is immunoprecipitated with a monoclonal antibody directed against ICP8 (L. Rafield and D. Knipe, manuscript in preparation). With the ICP8 mRNA probe, we observed hybridization to a major transcript of 4.5 kb as well as with several minor transcripts. The amount of all these transcripts was greater in cells infected with *ts*13 at 39.5°C than in cells infected with the ts^+ virus. At 33°C, the amount of these transcripts was greater in cells infected with the ts^+ virus (Fig. 5). Similarly, the amounts of the 6.6-kb transcript detected with the ICP5 mRNA probe and the 4.7-kb transcript detected with the ICP4 mRNA probe were greater in cells infected with *ts*13 at 39.5°C (Fig. 5). With the ICP4 mRNA probe, we detected very low quantities of the 3.0-kb mRNA that encodes ICP0 in RNA isolated from cells infected with *ts*13 or ts^+ virus. Therefore, we could not determine whether this mRNA was also overproduced. When we compared the levels of cytoplasmic polyadenylated transcripts isolated from these cultures with these same probes, similar results were obtained (P. J. Godowski and D. Knipe, data not shown). Thus, mutations in the major DNA-binding protein result in an increased steady state level of the mRNA for ICPs 4, 5, and 8.

DISCUSSION

We have shown that cells infected with certain HSV-1 group 1-1 mutants exhibit increased levels of viral gene expression. The members of this group contain mutations that map in or near the coding sequences of the major DNA-binding protein. These mutations lead to alterations in the cellular localization, DNA binding activities, or antigenic properties of the major DNA-binding protein (20, 36). It therefore seems likely that the alterations in viral gene expression are a result of the defect in the DNA-binding protein.

As reported previously, ICP8 exerts a positive effect on late gene expression, probably by promoting viral DNA replication (6). However, when viral DNA replication was inhibited in all infected cultures by incubation with PAA, the DNA-binding protein inhibited the expression of representative immediate early (ICP4), early (ICPs 6, 8, and 36), and late (ICPs 5, 11, and 15) proteins. Thus, under these conditions ICP8 acted as a negative regulator of viral gene expression.

We measured the rates of protein synthesis in infected cells by determining the amount of incorporation of [^{35}S]methionine into viral polypeptide bands during a short pulse period. The altered levels of viral gene expression observed in cells infected with ICP8 mutants probably reflect true differences in the amount of synthesis of these proteins because (i) viral proteins accumulated to higher levels in these cells as determined by Coomassie blue staining of the gels (data not shown), and (ii) the level of cytoplasmic viral mRNA was increased in these cells. Thus, the differences in labeling of polypeptides are probably not explained by trivial differences such as the uptake of label from the extracellular medium.

Role of ICP8 in the cascade regulation of HSV

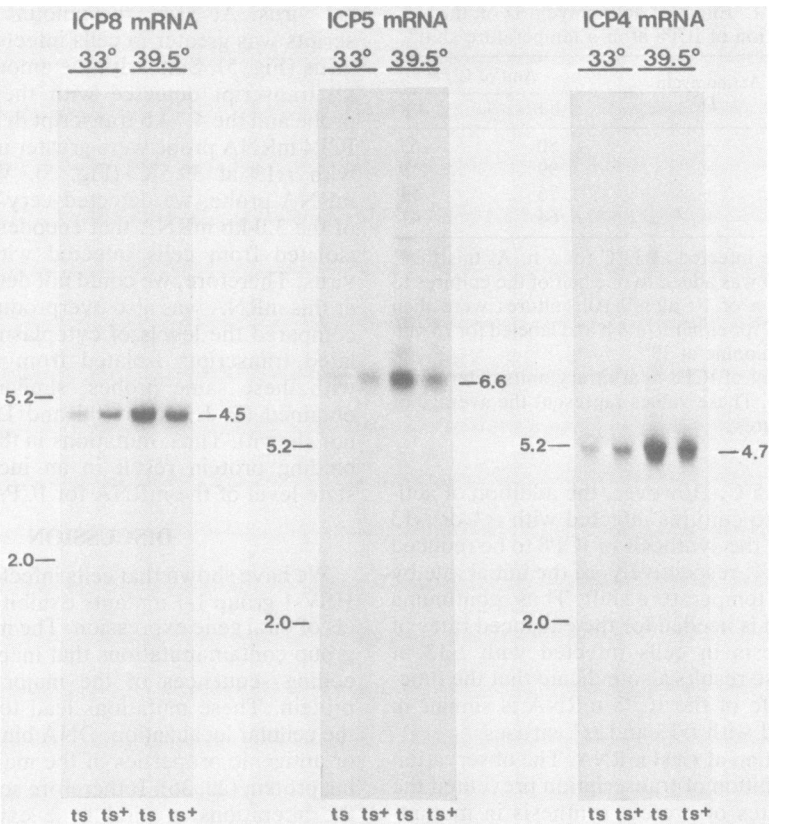

FIG. 5. Northern blot analysis of specific transcripts from cells infected with wild-type or mutant virus. Samples of total cytoplasmic RNA from cells infected with *ts*13 (ts) or KOS1.1 (ts^+) at 33 or 39.5°C were subjected to electrophoresis in formaldehyde-agarose gels and transferred to nitrocellulose as described in the text. To detect ICP8 mRNA, 20-μg samples of RNA on the blot were probed with ^{32}P-labeled pSG18SalA DNA. Autoradiography was performed at room temperature. To detect ICP5 mRNA or ICP4 mRNA, 10-μg samples of RNA on blots were probed with labeled *Bam*HI A′ DNA or *Bam*HI SP DNA, respectively. The size of the major RNA species detected with each probe is indicated in kilobases on the right of each panel. These sizes were determined relative to migration of 28S (5.2 kb) and 18S (2.0 kb) rRNA markers indicated on the left of each panel.

gene expression. In their model for cascade regulation of HSV gene expression, Honess and Roizman (13, 14) proposed that early proteins act as negative regulators of immediate early gene expression. We report evidence here that the ICP8 gene function affects the level of expression of ICP4, an immediate early protein. Thus, this is the first early gene function to be identified that regulates the immediate early to early gene transition.

ICP8 is needed for viral DNA replication and late gene expression. However, ICP8 inhibits late gene expression from parental viral genomes when viral DNA replication cannot occur. The late genes encode the majority of the virion structural polypeptides (29). Thus, this function prevents the synthesis of virion structural proteins until progeny DNA molecules are available for encapsidation.

Mechanism of action of ICP8 on viral gene expression. We have shown that the amount of mRNA encoding ICPs 4, 5, and 8 is higher in cells infected with ICP8 mutants. Thus, the ICP8 function regulates the cytoplasmic accumulation of viral mRNAs. We did not detect a difference in the functional stability of viral mRNAs in cells infected with these mutants in the presence of actinomycin D. These data suggest that the ICP8 function regulates the transcription or metabolism of newly synthesized mRNA. Alternatively, this result could reflect a need for continuous synthesis of a product that acts together with ICP8 to control viral gene expression.

There are two general models to explain the effect of the ICP8 function on viral gene expression. In the first, ICP8 inhibits the expression of the immediate early genes. Lower levels of the immediate early proteins would then cause low-

er levels of expression of all the later viral gene products. In this model, ICP8 would affect its own expression only indirectly. In the second model, ICP8 inhibits the expression of the different viral genes by a common mechanism. This could be mediated by the binding of ICP8 to viral DNA or to mRNA transcripts, for example. In this model, ICP8 affects its own expression more directly. This second model is supported by the observation that after a temperature shift, the expression of several viral genes, including that encoding protein 4, increased coordinately. Either of the two models proposed could incorporate the observation that after a shift to 39.5°C of cells infected with *ts*HA1 or *ts*13, ICP8 is released from viral DNA (20).

Similar alterations in the level of viral gene expression are observed in strains of adenovirus that encode a defective 72K DNA-binding protein. This protein, like the HSV-1 ICP8, is essential for viral DNA replication (33) and has a high affinity for single-stranded DNA (30). The 72K protein plays a multifunctional role in controlling adenovirus gene expression. It represses the transcription of early region 4 (24) and has been implicated in controlling the stability of early viral mRNAs (3). Mutations that alleviate the block of adenovirus late gene expression in monkey kidney cells map in the viral gene for the 72K protein (17). The simian virus 40 T antigen, an essential protein for simian virus 40 DNA replication, also negatively regulates early viral transcription. Both of these events involve the binding of T antigen to specific sequences near the origin of replication (11, 32). Further studies are in progress to determine the precise mechanism by which ICP8 affects HSV gene expression.

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