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Mutations in the Activation Region of Herpes Simplex Virus Regulatory Protein ICP27 Can Be *trans* Dominant

IRENE L. SMITH,† ROSE E. SEKULOVICH,‡ MARY ANN HARDWICKE, AND ROZANNE M. SANDRI-GOLDIN*

Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, California 92717

Received 18 December 1990/Accepted 9 April 1991

The herpes simplex virus type 1 (HSV-1) immediate-early protein ICP27 is an essential regulatory protein which is required for virus replication. Transfection experiments have demonstrated that ICP27 along with the HSV-1 transactivators ICP4 and ICP0 can positively regulate the expression of some late HSV-1 target plasmids and can negatively regulate the expression of some immediate-early and early target plasmids. We previously showed that mutants defective in the activation of a late target plasmid mapped to the carboxy-terminal half of the protein, whereas mutants defective in the repression of an early target plasmid mapped within the C-terminal 78 amino acids of ICP27 (M. A. Hardwicke, P. J. Vaughan, R. E. Sekulovich, R. O'Conner, and R. M. Sandri-Goldin, J. Virol. 63:4590–4602, 1989). In this study, we cotransfected ICP27 activator and repressor mutants along with wild-type ICP27 plasmid to determine whether these mutants could interfere with the wild-type activities. Mutants which were defective only in the activation function were dominant to the wild-type protein and inhibited the activation of the late target plasmid pVP5-CAT, whereas mutants defective in the repressor function did not inhibit either the activation of pVP5-CAT or the repression of the early target plasmid pTK-CAT. Furthermore, cell lines which stably carried three different activator mutants were impaired in their ability to support the growth of wild-type HSV-1 strain KOS, resulting in virus yields 5- to 40-fold lower than in control cells. The defect in virus replication appeared to stem from a decrease in the expression of HSV-1 late gene products during infection as measured by steady-state mRNA levels and by immunoprecipitation analysis of specific polypeptides. These results indicate that ICP27 activator mutations specifically interfere with the activation function of the protein both in transfection and during infection. Moreover, these results suggest that the repressor region may be important for binding of the polypeptide, since mutations in this region did not interfere with the activities of wild-type ICP27 and therefore presumably could not compete for binding.

Inhibition of function of a wild-type gene product can be accomplished by the expression of a variant of the same product. It has been proposed by Herskowitz (18) that the mutation of regulatory proteins may yield products that exhibit a dominant negative phenotype because these proteins contain multiple functional sites that can be mutated independently. This has been shown to occur for a number of viral regulatory proteins, including human immunodeficiency virus *tat* and *rev* (23, 27, 29). In the case of herpes simplex virus type 1 (HSV-1), *trans*-dominant mutations have been described in two pivotal regulatory proteins, ICP4 (Vmw175) which is the major *trans* activator during HSV-1 infection (41), and VP16 (Vmw65, α-TIF), which activates expression of the viral immediate-early genes (12). Expression of these mutant proteins resulted in the inhibition of the corresponding wild-type protein's activities and in impaired viral growth (12, 41). The mechanism of inhibition was different for the ICP4 and VP16 mutants, however. The ICP4 mutant lacked both transactivation domains found in the wild-type protein but retained DNA-binding and dimerization activities, resulting in the formation of ICP4 heterodimers containing one mutant and one wild-type subunit. These heterodimers were conformationally altered and functionally inactive (41). The mechanism of action for the VP16 mutant was proposed to result from the formation of nonproductive VP16–cellular DNA-binding protein complexes which competed with wild-type VP16 complexes for sites of action (12).

Studies such as those described for ICP4 and VP16 have aided in revealing functional domains in these regulatory proteins and in elucidating some of the molecular interactions of these proteins. For this reason, we undertook the analysis of mutants in another HSV-1 immediate-early gene which encodes the regulatory protein ICP27. ICP27 is a 63-kDa polypeptide which localizes to the nuclei of infected cells (1, 10, 20, 30) and is essential for HSV-1 growth (24, 34, 36). Studies with ICP27 temperature-sensitive (*ts*) and deletion mutants have demonstrated that ICP27 is involved in the negative regulation of immediate-early and some early genes and is required for the expression of later classes of genes (24, 34, 36). In transfection experiments, ICP27 appears to have little or no effect on its own on most HSV-1 target genes (7, 9, 40); however, in combination with the HSV-1 *trans* activators ICP4 and ICP0, ICP27 has been found to act as a *trans* activator on some late target genes and as a *trans* repressor on several immediate-early and early target genes (4, 9, 17, 26, 33, 35, 40, 43). These results indicate that ICP27 is involved in the switch from early to late gene expression during HSV-1 infection.

We previously constructed a series of in-frame insertion and deletion mutants (17), which were tested for their activities in transfection assays, to map the repressor and activator domains within the ICP27 polypeptide. The repres-

* Corresponding author.
† Present address: Department of Microbiology, State University of New York, Stony Brook, NY 11790.
‡ Present address: Chiron Corporation, Emeryville, CA 94608.

sor activity of ICP27 mapped to the carboxy-terminal 78 amino acids, whereas the activation function mapped to the carboxy-terminal half of the molecule encompassing a region of about 250 amino acids. To determine whether any of the ICP27 insertion mutants would display a dominant phenotype, we tested the mutant plasmids in cotransfection experiments with the wild-type plasmid on the expression of an HSV-1 early target gene and on an HSV-1 late target gene. All transfections were performed in the presence of ICP4 and ICP0. Mutants in the activation region, but not in the repressor region, were found to display a dominant negative phenotype in that these mutants interfered with the activation of the HSV-1 late target gene by the wild-type protein. In addition, cell lines stably transformed with three different dominant mutants were impaired in their ability to support the growth of wild-type HSV-1, resulting in virus yields ranging from 5- to 30-fold lower than those measured in control cells. Analysis of several viral mRNAs and proteins from infections of these dominant mutant-transformed cells showed a significantly lower expression of HSV-1 late gene products. Viral DNA replication in these cell lines was comparable to or only slightly lower than that seen in control cells, indicating that the reduction in late gene expression was not due to an inhibition of DNA synthesis. These results demonstrate that mutants which are defective in the activation function of ICP27, but not the repressor activity, are dominant to the wild-type protein and specifically interfere with its activation function both in transfection and in infection.

MATERIALS AND METHODS

Bacteria and plasmids. *Escherichia coli* K-12 strain 1100 derivative DH-1 (16) was used as the host for the propagation of all chimeric plasmids. The target plasmids pTK-CAT and pVP5-CAT were described previously (40). The effector plasmids pSG28K/B (which contains the ICP4 gene), pRS-1 (which contains the ICP0 gene), and pSG130B/S (which contains the wild-type ICP27 gene) have been described previously (40). The construction and characterization of the ICP27 insertion mutant plasmids S13, N21, S23, S1B, B7, N2, S2, and S18 have been described (17), as has the cloning of plasmid LG4 (17). Briefly, mutant S13 has a four-amino-acid insertion between residues 262 and 263; N21 has a four-amino-acid insertion at residue 327; S23 has a two-amino-acid insertion at residue 383 as well as a substitution of a glycine for an alanine at this position; S1B contains a four-amino-acid insertion at residue 406; B7 has four amino acids inserted at residue 434; N2 has a substitution of two arginine residues for a methionine and a histidine at residues 459 and 460 as well as an insertion of two serines; S2 has four amino acids inserted at residue 465; and S18 has a substitution of a serine for the tyrosine at position 504 and an insertion of four amino acids after the substitution. The ICP27 frameshift mutant R2 was constructed by digestion of plasmid pSG130B/S with *Rsr*II at nucleotide position 584 relative to the *Bam*HI site of this plasmid, which is designated nucleotide position 1 (see Fig. 1). The 3-bp 5′ overhang was filled in with Klenow enzyme, and a synthetic oligonucleotide, GGAATTCC, encoding an *Eco*RI site was ligated to the plasmid. This resulted in the net addition of 11 bp, causing a frameshift at amino acid 59 in the ICP27 polypeptide. The mutant was sequenced around the site of the linker insertion to confirm the 11-bp addition.

Cells, viruses, and transfection. Rabbit skin fibroblasts (RSF) from the American Type Culture Collection were grown as described previously (40). HSV-1 strain KOS 1.1, the ICP27 temperature-sensitive mutant virus *ts*LG4, and HSV-2 strain HG52 were propagated and assayed as described previously (38). For transfection experiments in which chloramphenicol acetyltransferase (CAT) activity was assayed, transfections were performed in 35-mm-diameter six-well cluster dishes as described previously (40). The target plasmids were added at 20 μg of plasmid DNA per well, whereas the effector plasmid pSG28K/B was added at 5 μg per well and pRS1 was added at 2.5 μg per well. The wild-type ICP27 plasmid, pSG130B/S, and the insertion mutant plasmids were added at different ratios as described in the figure legends. Cells were harvested 48 h after transfection. CAT activity was measured by the diffusion assay of Neumann et al. (28), modified as described previously (17).

For the transfection experiments measuring the infectivity of HSV-1 KOS DNA, RSF cells were transfected with 5 μg of KOS DNA per ml. Plasmid DNA was added at a concentration of 1 μg/ml. The transfected cultures were overlaid with methylcellulose 24 h after transfection and incubated at 37°C until plaques were clearly visible. This occurred within 5 to 6 days. Plaques were stained with crystal violet. Marker rescue experiments to map the location of the lesion in *ts*LG4 were performed as described previously (13).

For the isolation of cell lines containing the ICP27 mutant plasmids, RSF cells were transfected with 5 μg of S23, S1B, N2, or LG4 plasmid DNA and 1 μg of pFeLTR-neo, which contains the gene encoding resistance to G418 under the control of the feline leukemia virus long terminal repeat as described earlier (39). Selection in medium containing 750 μg of G418 per ml was initiated 24 h after transfection. Colonies which were clearly visible after 3 weeks were picked and expanded. G418-resistant colonies were initially screened for their ability to plaque wild-type HSV-1 KOS. Those cell lines which exhibited at least a fivefold-lower efficiency of plaquing KOS were screened for the presence of ICP27 sequences by Southern blot hybridization of cellular DNA as described previously (37). Several cell lines containing wild-type ICP27 sequences or the repressor mutant N2 which did not lower virus plaquing efficiency were also screened for the presence of ICP27 sequences by Southern blot hybridization.

Measurement of DNA replication. Cell lines were infected with HSV-1 KOS at a multiplicity of infection of 1.0 in 60-mm-diameter dishes. At 1 and 12 h after infection, infected cultures were harvested and total DNA was isolated as described previously (37). Purified DNA was denatured and applied to nitrocellulose filters, using a slot blot apparatus (Bethesda Research Laboratories), at the concentrations designated in Fig. 7. The blots were hybridized with a ^{32}P-labeled probe specific for the gC gene, which was nick translated and denatured as described previously (42). Parallel samples were incubated until 24 h after infection, at which time infected cultures were harvested and virus yields were assayed on RSF cells.

Northern (RNA) hybridizations. Total RNA was extracted by the guanidium thiocyanate method (6). The concentration of the RNA samples was estimated by ethidium bromide staining of a fraction of each sample after agarose gel electrophoresis. Equivalent amounts of total RNA from each cell line were subsequently denatured in glyoxal (44) and fractionated in 1% agarose gels. Transfer of the RNA to GeneScreen (Du Pont, NEN Research Products) and hybridization conditions were as described previously (37). ^{32}P-labeled probes were nick translated and denatured as described previously (37). Hybridized probe was removed

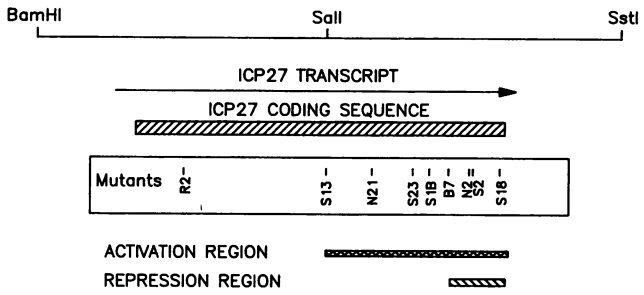

FIG. 1. Schematic representation of the ICP27 gene showing the positions of several insertion mutants. The ICP27 gene of HSV-1 is contained within a 2,421-bp *Bam*HI-to-*Sst*I fragment in the unique long region of the genome between coordinates 0.745 and 0.765 (25). In this schematic representation, the *Bam*HI site has been designated nucleotide 1 and the *Sst*I site has been designated nucleotide 2421. The *Bam*HI-to-*Sst*I fragment was cloned into pUC18 and designated pSG130B/S (40). The positions of the insertions in mutants S13, N21, S23, S1B, B7, N2, S2, and S18 (17) and the frameshift mutant R2 are shown. The positions of the 1.8-kb unspliced transcript which initiates at nucleotide 275 (21, 22, 47) and the ICP27 coding region which initiates at an AUG at nucleotide 413 and terminates at 1949 (25) are also shown.

before rehybridization with different probes by two successive washes in 0.05× wash buffer (1× wash buffer contains 50 mM Tris [pH 8.0], 2 mM EDTA, 0.5% PP_i, and 0.02% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone–50% formamide) (44) for 90 min each at 80°C with constant agitation.

Immunoprecipitation and polyacrylamide gel electrophoresis. Immunoprecipitations with monoclonal antibodies to gD and gC were performed as described previously (42). The antibodies were generously provided by J. Glorioso (19). Polyacrylamide gel electrophoresis was performed as described by Smith and Sandri-Goldin (42).

RESULTS

Mutants of ICP27 which are defective in the activation function but not the repressor activity are *trans*-dominant to the wild-type protein in cotransfections. We previously showed that ICP27 insertion mutants which map between amino acids 260 and 434 were defective in the ability to stimulate the expression of the HSV-1 late target plasmid pVP5-CAT above the level seen with ICP4 and ICP0 in cotransfection experiments (17). These mutants are termed activator mutants. We further showed that insertion mutants mapping between amino acids 434 and 508 in the carboxy terminus of the ICP27 polypeptide were defective both in the activation function and in the ability to repress expression of the early target plasmid pTK-CAT from the level seen with ICP4 and ICP0 (17). These mutants, while defective in both functions, are termed repressor mutants here to distinguish them from the activator mutants. To learn more about the functional domains of ICP27, we tested each of the activator insertion mutants and the repressor insertion mutants in transfection experiments with the wild-type ICP27 plasmid to determine whether either activity could be inhibited by the mutant proteins. Figure 1 shows the positions of the mutations in the ICP27 gene. The activator mutants S13, N21, S23, and S1B and the repressor mutants B7, N2, S2, and S18 have been fully described (17). The frameshift mutant R2 was constructed as a control for these experiments as described in Materials and Methods. This mutant

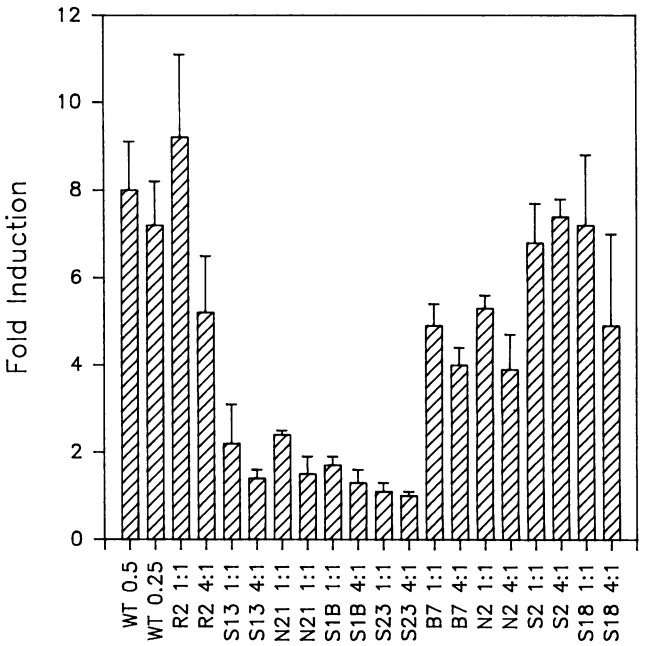

FIG. 2. Dominant negative effect of ICP27 activation mutants on the ability of wild-type ICP27 to augment the expression of the HSV-1 target plasmid pVP5-CAT. The target plasmid pVP5-CAT (20 μg/ml) was cotransfected with the effector plasmids pSG28K/B (ICP4; 5 μg/ml) and pRS1 (ICP0; 2.5 μg/ml) in all panels. Plasmid pSG130 B/S containing the wild-type (WT) ICP27 gene was added to the transfections at a concentration of 0.5 or 0.25 μg/ml, as indicated. Mutant plasmids R2, S13, N21, S1B, S23, B7, N2, S2, and S18 were added to the transfections along with wild-type ICP27 plasmid at a ratio of 1:1 (0.5 μg of mutant plasmid and 0.5 μg of wild-type plasmid per ml) or 4:1 (1.0 μg of mutant plasmid and 0.25 μg of wild-type plasmid per ml), as indicated. The data are represented as fold induction relative to the induction seen with the ICP4 and ICP0 plasmids in the absence of ICP27. The value for the induction obtained with ICP4 and ICP0 was derived from 10 separate transfection experiments. The mean value for the fold induction with ICP4 and ICP0 above the constitutive level of expression seen with uninduced pVP5-CAT was 3.5 ± 1.6. This value was then arbitrarily set equal to 1.0, and fold induction with wild-type ICP27 alone and in combination with the insertion mutants was calculated. Each transfection was performed at least six times to control for variability. Error bars are shown.

encodes only the first 58 amino acids of the 512-amino-acid polypeptide and is defective in both the activator and repressor functions (data not shown). When wild-type ICP27 plasmid was added to transfections with pVP5-CAT in the presence of ICP4 and ICP0, the level of CAT expression was seven- to ninefold higher than the level found with ICP4 and ICP0 alone (Fig. 2). As reported earlier (17), none of the activator or repressor mutants was capable of activating pVP5-CAT expression (data not shown). When the activator mutant S13, N21, S1B, or S23 was added to the transfections along with the wild-type ICP27 plasmid at a 1:1 ratio, the level of stimulation was three- to fivefold lower than the level seen with the wild-type plasmid alone, and this effect was even greater at the 4:1 ratio of mutant to wild-type plasmid (Fig. 2). A similar decrease in activation was not found in the cotransfections with the repressor mutant B7, N2, S2, or S18 or with the frameshift mutant R2. These data suggest that the activator mutants of ICP27 can act in a dominant manner to the wild-type protein.

TABLE 1. Effects of ICP27 repressor mutants on the repressor activity of the wild-type protein

Effector plasmid[a]	Mean pTK-CAT expression[b] ± SEM
Uninduced	1.0
ICP4 + ICP0	20.9 ± 5.5
ICP4 + ICP0 + ICP27 (0.5 μg)	1.2 ± 0.5
ICP4 + ICP0 + ICP27 (0.25 μg)	1.1 ± 0.4
ICP4 + ICP0 + ICP27 (0.5 μg) + R2 (0.5 μg)	2.3 ± 1.1
ICP4 + ICP0 + ICP27 (0.25 μg) + R2 (1.0 μg)	1.7 ± 0.9
ICP4 + ICP0 + ICP27 (0.5 μg) + B7 (0.5 μg)	1.1 ± 0.2
ICP4 + ICP0 + ICP27 (0.25 μg) + B7 (1.0 μg)	1.9 ± 0.6
ICP4 + ICP0 + ICP27 (0.5 μg) + N2 (0.5 μg)	2.0 ± 1.1
ICP4 + ICP0 + ICP27 (0.25 μg) + N2 (1.0 μg)	1.8 ± 0.9
ICP4 + ICP0 + ICP27 (0.5 μg) + S2 (0.5 μg)	2.5 ± 0.8
ICP4 + ICP0 + ICP27 (0.25 μg) + S2 (1.0 μg)	1.9 ± 1.1
ICP4 + ICP0 + ICP27 (0.5 μg) + S18 (0.5 μg)	2.6 ± 0.4
ICP4 + ICP0 + ICP27 (0.25 μg) + S18 (1.0 μg)	3.4 ± 0.3

[a] Plasmid pSG28K/B containing the ICP4 gene was added to the transfections at 5 μg/ml, and pRS1 containing the ICP0 gene was added at 2.5 μg/ml. Plasmid pSG130B/S containing the wild-type ICP27 gene was added at the concentrations indicated, as were the plasmids containing the ICP27 repressor mutants.

[b] Values from three experiments are presented as fold induction over the uninduced level obtained with pTK-CAT in the absence of effector plasmids. This value was set equal to 1.0.

We tested this possibility further by measuring activation of pVP5-CAT expression in the presence of wild-type ICP27 and mutant LG4, another activator mutant. The ICP27 gene in plasmid LG4 was cloned from the temperature-sensitive HSV-1 ICP27 mutant *ts*LG4 (38). Mutant plasmid LG4 displayed a temperature-sensitive activator phenotype in that it failed to activate pVP5-CAT at the nonpermissive temperature of 39°C but did so at 34°C (17). It was able to repress expression of the early target plasmid pTK-CAT at both temperatures (17). Mutant LG4 was cotransfected with wild-type ICP27 plasmid at 1:1 and 4:1 ratios at 39°C, and pVP5-CAT expression was measured by CAT assay 48 h later. Wild-type ICP27 alone stimulated CAT expression an average of 4.5 ± 1.4-fold at 0.5 μg/ml and 7.1 ± 2.4-fold at 0.25 μg/ml above the level seen with ICP4 and ICP0 in six separate transfection experiments. Addition of plasmid LG4 to the transfections with wild-type ICP27 resulted in fold induction of 1.2 ± 0.3 (1:1) and 0.9 ± 0.2 (4:1) above the level seen with ICP4 and ICP0. Again, these data represent values from six separate transfections. Therefore, LG4, a temperature-sensitive activator mutant, is also a temperature-sensitive *trans*-dominant mutant.

To test whether the repressor mutants were dominant for the repressor-negative phenotype, mutants B7, N2, S2, and S1B as well as frameshift mutant R2 were cotransfected with wild-type ICP27 and the early gene target plasmid pTK-CAT. The activator mutants were not used in these transfections because these mutants repress pTK-CAT expression as efficiently as does wild-type ICP27 (17). As shown in Table 1, none of the repressor mutants interfered with the repression of pTK-CAT expression by the wild-type protein. Therefore, the repressor mutants of ICP27 are not dominant to the wild-type phenotype in either the activation or repression functions, and the *trans*-dominant mutants of ICP27 map to the activation domain between amino acids 260 and 434.

ICP27 *trans*-dominant mutants reduce the infectivity of HSV-1 KOS DNA. To determine whether the ICP27 *trans*-dominant activator mutants could interfere with virus production during infection, we cotransfected infectious KOS DNA with plasmids encoding the ICP27 activator mutants and monitored the production of viral plaques. Cultures were overlaid with methylcellulose 24 h after transfection, and plaques were stained and counted 6 days later. The numbers of plaques produced in the absence of added plasmid were 650 and 544 in two separate transfections (Table 2). Addition of ICP0 plasmid did not lower the number of plaques produced; however, addition of the wild-type ICP27 plasmid resulted in a reduction of nearly fivefold. It is possible that the high level of expression of ICP27 during infection, which would have resulted from the combined expression of the viral ICP27 as well as that from the plasmid, had a deleterious effect on HSV-1 growth, perhaps because of the repressor activity of the protein. This is further suggested by the result that transfections with mutant N2 or S2, each of which is defective in the repressor function, yielded nearly as many plaques as those with no ICP27 plasmid added (Table 2). However, in comparison, the transfections with the dominant activator mutants S13, N21, S23, and S1B showed a 50- to 500-fold reduction in the number of plaques produced compared with transfections with no plasmid added and a 10- to 100-fold reduction relative to transfections with the wild-type ICP27 plasmid. Therefore, the dominant mutants are capable of interfering with HSV-1 productive infection to a greater extent than can the wild-type plasmid, which shows some inhibition.

TABLE 2. Effects of ICP27 *trans*-dominant mutants on the infectivity of HSV-1 KOS DNA

Plasmid	PFU[a]
None	650, 544
Wild-type ICP0	590, 493
Wild-type ICP27	162, 98
S13	0, 3
N21	12, 10
S23	2, 0
S1B	21, 2
N2	523, 325
S2	432, 390

[a] Values represent two separate transfections for each plasmid. Plasmid DNA was added at a concentration of 1 μg/ml. Viral DNA was added at a concentration of 5 μg/ml.

Isolation and characterization of cell lines containing ICP27 dominant mutants. Next, we wished to characterize the inhibition of virus growth by the ICP27 dominant mutants. To do so, cell lines containing three different dominant mutants were isolated to have sufficient material to monitor specific HSV-1 products throughout infection. RSF cells were cotransfected with plasmid, pFeLTR-neo, which contains the neomycin resistance gene (39), and either wild-type ICP27 plasmid, the repressor mutant N2, or the dominant mutant S23, S1B, or LG4. G418-resistant colonies were selected and expanded. Cell lines transformed with the dominant mutants were screened for their ability to support the growth of HSV-1, since the transfection results with KOS DNA presented earlier suggested that expression of the dominant mutants would interfere with virus growth. Over 20 independently isolated cell lines were screened for each mutant. Figure 3 shows the results of two experiments performed with cell line 23-5, which was transformed with the *ts* dominant mutant LG4. In Fig. 3A, a stock of KOS was assayed on parental RSF cells and on cell line 23-5 at either 34 or 39°C. The efficiency of plaquing of KOS on 23-5 cells at the nonpermissive temperature was reduced more than

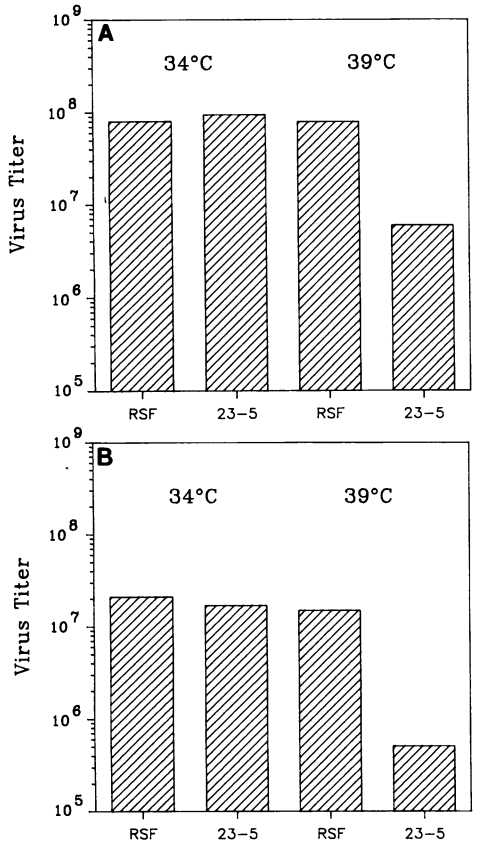

FIG. 3. Efficiency of growth of wild-type HSV-1 on a cell line containing a temperature-sensitive *trans*-dominant ICP27 mutant. The G418-resistant cell line 23-5, which contains the ICP27 *ts* allele from mutant *ts*LG4, was tested for its ability to propagate the growth of wild-type HSV-1 KOS. To measure plaquing efficiency (A), a stock of KOS was diluted and plated on 23-5 cells and on the parental cell line RSF at either 34 or 39°C. Plaques were counted after 6 days of incubation. To measure infectious virus yield (B), each cell line was infected with KOS at a multiplicity of infection of 1.0, and infected cultures were incubated at 34 or 39°C for 24 h. Cell lysates were assayed for infectious virus yields on RSF cells at 34°C.

10-fold. Similar results (at 37°C) were found for cell lines Stu1, Stu8, and Stu15, which were transformed with S1B, and for Sst26, which was transformed with S23 (data not shown). These cell lines were chosen for further study.

To test the production of infectious virus in 23-5 cells, infections with KOS at a multiplicity of infection of 1.0 were performed at 34 and 39°C. Twenty-four hours later, infected cultures were harvested and virus yields were assayed on RSF cells at 34°C. The yield of virus produced in 23-5 cells at 34°C was equivalent to that produced in RSF cells; however, there was a reduction of approximately 40-fold in the virus yield from infections of 23-5 cells at 39°C (Fig. 3B). These results indicate that 23-5 cells are not generally toxic for HSV-1 infection because virus growth was efficiently supported at the permissive temperature. Rather, the LG4 mutation, which behaved in a *ts* dominant manner in the transfections with pVP5-CAT, appears to be behaving in the same manner in the 23-5 cell line. That is, the mutant protein is dominantly interfering with infection only at the nonpermissive temperature.

A similar experiment to measure virus yields was performed with cell lines Stu1, Stu8, Stu15, and Sst26. These cell lines as well as the control RSF cells, cell line 39, which was transformed with the wild-type ICP27 plasmid, and Nsi37, which was transformed with the repressor mutant N2, were infected with KOS at a multiplicity of infection of 0.2, 2, or 10 (Table 3). Virus yields were assayed on RSF cells 24 h after infection. There was a 5-fold (Stu8) to 30-fold (Sst26) reduction in virus yield from infections of the dominant mutant cell lines at the lowest multiplicity (Table 3). Yields were higher when the multiplicity was raised to 2 or 10, so that differences seen between the dominant mutant cell lines and the control cell lines were only about two- to sixfold. This result is consistent with the hypothesis that the dominant mutant protein expressed by the cell line is competing with the virally expressed wild-type ICP27. A gene dosage effect was observed as more copies of the wild-type gene were added at the higher multiplicity of infection.

TABLE 3. Measurement of yield of HSV-1 KOS 1.1 on cell lines stably transformed with ICP27 mutants[a]

Cell line	Yield at multiplicity of infection of:		
	0.2	2	10
RSF	1.9×10^7	4.1×10^7	5.5×10^7
39	1.8×10^7	4.8×10^7	7.4×10^7
Stu1	3.3×10^6	6.5×10^6	1.2×10^7
Stu8	4.1×10^6	2.0×10^7	4.2×10^7
Stu15	8.0×10^5	6.3×10^6	6.8×10^6
Sst26	5.9×10^5	1.2×10^7	1.3×10^7
Nsi37	2.9×10^7	6.1×10^7	4.7×10^7

[a] The parental cell line (RSF) and cell lines containing either the wild-type ICP27 gene (39) or mutants S1B (Stu1, Stu8, and Stu15), S23 (Sst26), and N2 (Nsi37) were infected with wild-type strain KOS at 37°C for 24 h. Virus yields were assayed on RSF cells.

Again, to be certain that these cell lines were not generally toxic for HSV infection, HSV-2 wild-type strain HG52 was plated on cell lines Stu15, Sst26, and Nsi37 and on parental RSF cells, and their efficiency of plating was compared with that of HSV-1 KOS. Table 4 shows that the efficiency of plaquing HSV-2 on Sst26 cells was equivalent to that seen on RSF and Nsi37 cells, whereas the number of HSV-1 plaques was reduced sixfold. Similarly, the number of HSV-1 plaques was about 14-fold lower in Stu15 than in RSF cells, yet the number of HSV-2 plaques was reduced only about threefold. These data indicate that the mutant ICP27 protein in these cell lines interferes with HSV-1 growth and that these cell lines do not generally block HSV replication.

Southern blot hybridization analysis of DNA from cell lines transformed with ICP27 dominant mutants. While the cell

TABLE 4. Comparison of efficiencies of plating of HSV-2 HG52 and HSV-1 KOS on cell lines stably expressing ICP27 mutants[a]

Cell line	PFU	
	HSV-1	HSV-2
RSF	4.8×10^7	7.2×10^7
Stu15	3.5×10^6	2.5×10^7
Sst26	8.1×10^6	6.2×10^7
Nsi37	3.3×10^7	5.3×10^7

[a] A dilution of wild-type HSV-2 strain HG52 or HSV-1 KOS was plated on the parental cell line RSF and on cell lines containing ICP27 mutants S1B (Stu15), S23 (Sst26), and N2 (Nsi37). Monolayers were overlaid with methylcellulose, and plates were incubated at 37°C for 6 days, at which time plaques were stained and counted.

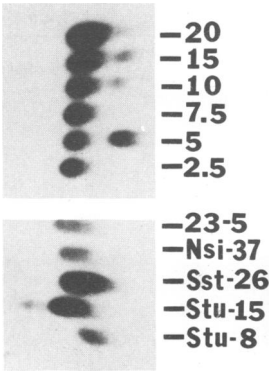

FIG. 4. Southern blot hybridization analysis of DNA from cell lines transformed with ICP27 *trans*-dominant mutants. DNA (10 μg) from cell lines 23-5, Nsi37, Sst26, Stu15, and Stu8 was digested with *Bam*HI and *Sst*I, fractionated in 1% agarose, and then transferred to a GeneScreen Plus membrane. RSF DNA (10 μg per sample) was mixed with plasmid pSG130B/S to approximate 2.5 (25 pg/10 μg), 5 (50 pg), 7.5 (75 pg), 10 (100 pg), 15 (150 pg), and 20 (200 pg) copies per cell and were then similarly digested and fractionated. The blot was hybridized with ^{32}P-labeled *Bam*HI-*Sst*I fragment from the wild-type ICP27 plasmid pSG130B/S.

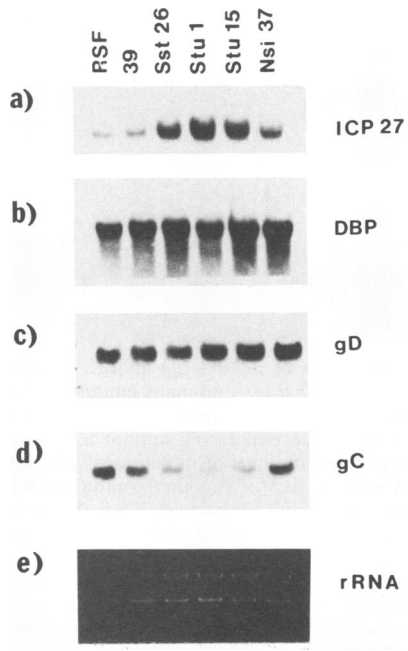

FIG. 5. Northern blot analysis of steady-state levels of specific HSV-1 mRNAs in cells expressing *trans*-dominant ICP27 mutants. Parental RSF cells or cells containing the wild-type ICP27 gene (39), the *trans*-dominant mutant S23 (Sst26) or S1B (Stu1 and Stu15), or the repressor mutant N2 (Nsi37) were infected with KOS at a multiplicity of infection of 0.5. Total RNA was extracted 5 h after infection. A fraction of each RNA sample was electrophoresed in agarose and stained with ethidium bromide to determine that equivalent amounts of RNA from each cell line were being analyzed. The stained gel showing the 28S and 18S rRNA bands is shown in panel e. Equivalent amounts of RNA for each sample were then denatured in glyoxal, fractionated in 1% agarose, and transferred to GeneScreen. The blot in panel a was hybridized with a ^{32}P-labeled 1,200-bp *Bam*HI-*Sal*I DNA probe specific for ICP27 mRNA (see Fig. 1). Panel b was hybridized with a labeled 750-bp *Bam*HI-*Xho*I fragment from the gene encoding the major DNA-binding protein ICP8. After autoradiography, the blot in panel a was stripped of the hybridized probe and rehybridized with a ^{32}P-labeled 846-bp *Hin*dIII-*Acc*I fragment from the gD gene (panel c). The blot in panel b was rehybridized with a 960-bp *Eco*RI-*Xba*I fragment from the gC gene. The isolation and labeling of the DNA fragments as well as their locations within each gene were described previously (42).

lines transformed with the dominant mutants inhibited HSV-1 growth as proposed, it was necessary to confirm that these cell lines contained ICP27 sequences. Therefore, Southern blot hybridizations were performed on DNA samples extracted from the cell lines. In addition, to ascertain the number of copies per cell line, standards were set up in which pSG130B/S plasmid DNA was mixed with RSF DNA to approximate 2.5, 5, 7.5, 10, 15, and 20 copies per cell (Fig. 4). All samples were digested with *Bam*HI and *Sst*I. The intensity of the 2.4-kb *Bam*HI-*Sst*I band which hybridized with the ICP27-specific probe in each cell line was compared with the intensity of hybridization in the standards. Cell lines 23-5, containing the *ts* mutant LG4, Nsi37, containing the repressor mutant N2, and Stu8, containing the dominant mutant S1B, appeared to have around one complete copy of the ICP27 fragment, whereas Sst26 (S23 mutant) and Stu15 (S1B) contained multiple copies, in the range of 10 to 15.

Expression of specific HSV-1 products during infection of cell lines containing dominant negative mutants. To determine whether the inhibition of HSV-1 growth in the ICP27 dominant mutant cell lines occurred because of targeted interference with ICP27 function during infection, we examined the steady-state mRNA levels of HSV-1 genes from different kinetic classes in cell lines infected with KOS. It was postulated that since the dominant negative mutants were those which were defective in the activation function, then the activation of late gene expression during HSV-1 infection should be specifically inhibited by these mutants, whereas repressor activities would not be affected. Cell lines Sst26, Stu1, and Stu15 as well as control RSF cells, cell line 39, and Nsi37 were infected with KOS at a multiplicity of infection of 0.5. Total RNA was isolated 5 h after infection. To ensure that equivalent amounts of RNA were being analyzed from each cell line, an aliquot from each sample was fractionated in agarose and stained with ethidium bromide (Fig. 5e). Northern blot hybridization was performed on equivalent samples from each cell line. In Fig. 5a, the blot was hybridized with a probe specific for ICP27 mRNA. The higher expression of ICP27 in cell lines 39, Sst26, Stu1, Stu15, and Nsi37 is likely due to the presence of the ICP27 sequences resident in these cell lines. In all cases, the ICP27 gene is under its own promoter so that expression in the absence of infection is not detectable (unpublished results). This has also been shown for other cell lines containing HSV-1 genes under their own promoters (7, 24, 37). However, infection with HSV-1 results in the induction of the resident ICP27 gene by VP16 (5, 22, 32), confirming that these sequences are being expressed in these cells. The level of ICP27 expression does not completely correlate with the copy number of the ICP27 sequences in these cells (Fig. 4). We have previously found the same result with other HSV-1-transformed cell lines (37).

In Fig. 5b, the blot was hybridized with a probe specific for the major DNA-binding protein (ICP8) mRNA. This early gene product was expressed at nearly equivalent levels in all of the cell lines, as was the transcript for gD, another early gene product (Fig. 5c). To obtain this result, the blot in Fig. 5a was stripped of the radioactive probe and rehybridized with a gD-specific probe. These results show that the

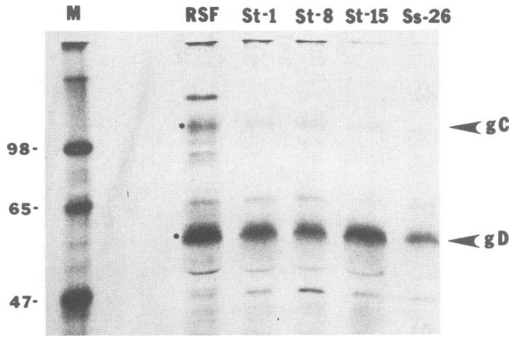

FIG. 6. Expression of HSV-1 gD and gC in KOS-infected cell lines containing ICP27 *trans*-dominant mutants. RSF cells and cell lines Stu1, Stu8, and Stu15 expressing the *trans*-dominant mutant S1B and cell line Sst26 expressing mutant S23 were infected with HSV-1 KOS at a multiplicity of infection of 1.0. Infected cultures were labeled with 50 μCi of [^{35}S]methionine per ml beginning 2 h after infection. Cultures were harvested 6 h after infection, and cell lysates were immunoprecipitated with a monoclonal antibody specific for gD, an HSV-1 early gene product (42), and with an antibody specific for gC, a late gene product (42). Labeling conditions, immunoprecipitation procedures, and polyacrylamide gel electrophoresis were as described previously (42). Positions of size markers (M) are shown in kilodaltons on the left.

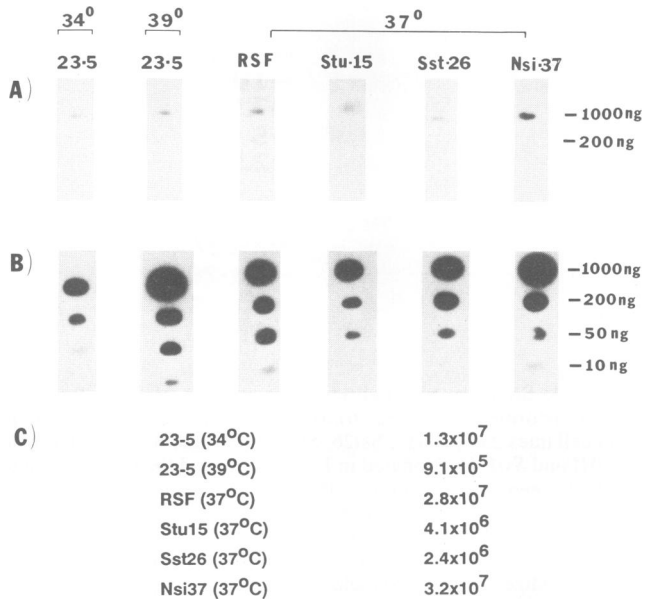

FIG. 7. Measurement of DNA replication in KOS-infected cell lines containing ICP27 *trans*-dominant mutants. RSF cells and cell lines Stu15, Sst26, and Nsi37 were infected with HSV-1 KOS at a multiplicity of infection of 1.0 at 37°C, and cell line 23-5 was similarly infected at 34 and 39°C. DNA was purified from infected cultures at 1 h after infection (A) and at 12 h after infection (B). Equivalent amounts of total DNA for each sample over a 100-fold concentration range were loaded onto nitrocellulose filters, using a slot blot apparatus. Blots were hybridized with a nick-translated DNA probe specific for the gC gene as described in the legend to Fig. 5. Parallel cultures were incubated for 24 h, at which time virus yields were measured by plaque assay on RSF cells (C).

expression of two early gene products was unaffected during infection of the cell lines containing the dominant mutants compared with the control cells. In contrast, when a probe specific for the late products of the gC gene was used to rehybridize the blot from Fig. 5b, a lower level of gC mRNA was found in the RNA samples from the dominant mutant cell lines Sst26, Stu1, and Stu15 than in the controls. Similar levels of gC mRNA were seen in the samples from the parental RSF cells, cell line 39, containing wild-type ICP27 sequences, and Nsi37, which contains the nondominant repressor mutant N2. Therefore, the Northern blot analysis showed that the dominant ICP27 mutants were being expressed in these transformed cells and furthermore, early gene expression was not affected during infection but late gene expression was reduced.

This was further shown by monitoring the expression of the early protein gD and the late protein gC in the transformed cell lines following infection with KOS. RSF cells and cell lines Stu1, Stu8, Stu15, and Sst26 were labeled with [^{35}S]methionine 2 h after infection, and cells were harvested 4 h later. Cell lysates were immunoprecipitated with a monoclonal antibody to gD and with a monoclonal antibody specific for gC (19). Figure 6 shows the autoradiogram of the polyacrylamide gel on which the samples were fractionated. Again, the amount of expression of the early gD product was not greatly affected in any of the cell lines, whereas expression of the late product gC was reduced in the cell lines containing ICP27 dominant mutants. A similar result was found with use of a monoclonal antibody to p40 (48), another HSV-1 late gene product, in that p40 expression was lower in cell lines Stu15 and Sst26 than in RSF cells at 37°C and in 23-5 cells at 39.5°C than in 23-5 cells at 34°C (data not shown).

ICP27 deletion mutants and some nonsense mutants display a fivefold reduction in viral DNA replication (24, 34), although several ICP27 *ts* mutants synthesize DNA in amounts nearly equivalent to those synthesized by wild-type KOS (34, 36). Therefore, ICP27 appears to have a role in DNA replication. Because HSV-1 late gene expression is dependent on DNA replication, we wanted to determine whether the decrease in late gene expression in the dominant cell lines was due to a direct interference with the activation function of wild-type ICP27 or to an indirect effect on viral DNA replication. Mutant containing cell lines and control RSF cells were infected with HSV-1 KOS at a multiplicity of infection of 1.0. DNA was isolated from infected cultures at 1 and at 12 h after infection. In addition, parallel infections were incubated for 24 h to monitor virus yields in this experiment. Equivalent DNA concentrations for each sample were applied to nitrocellulose filters by using a slot blot device as shown in Fig. 7. The filters were hybridized with a ^{32}P-labeled DNA probe specific for the gC gene (42). Appropriate exposures of the autoradiograms were scanned by a densitometer. DNA replication in Sst26-infected cells was only about 1.5-fold lower than in RSF-infected cells, whereas DNA replication in Stu15 cells was reduced less than 3-fold. Virus yields from Sst26-infected cells were 10-fold lower than in RSF cells; in Stu15 cells, yields were 7-fold lower. Therefore, although viral yields were substantially reduced in Sst26- and Stu15-infected cells, DNA replication was not comparably affected. The slight reduction in DNA replication, therefore, cannot account for the level of reduction seen in late gene expression. We conclude that the mutant ICP27 in these cell lines is directly interfering with the activator function of the wild-type protein and not with its role in DNA replication. This conclusion is further supported by the results with cell line 23-5 at 39.5 versus

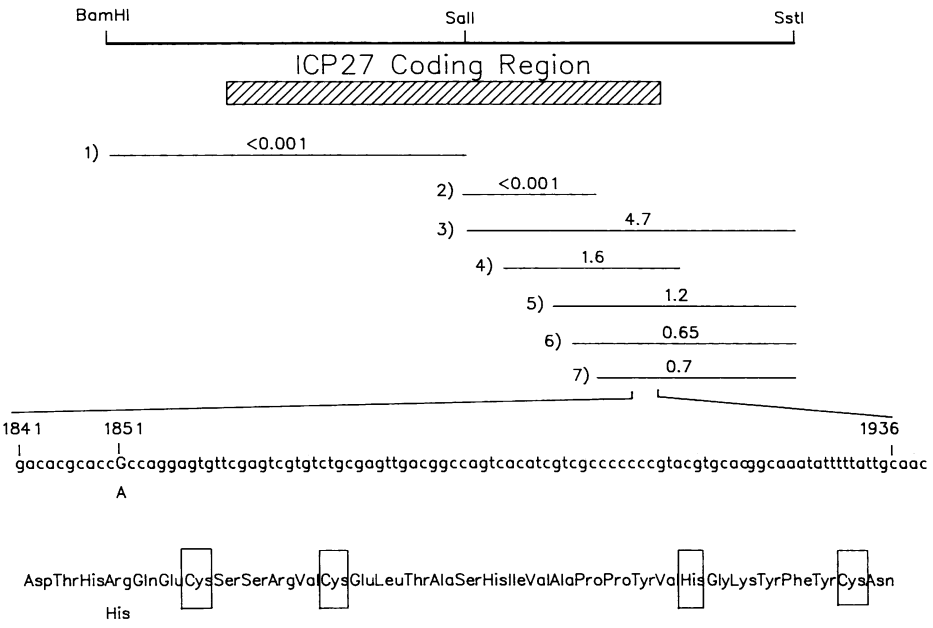

FIG. 8. Marker rescue and DNA sequence analysis of the *ts* lesion in HSV-1 *ts*LG4. Fragments from the wild-type ICP27 plasmid pSG130B/S were gel purified and mixed with viral *ts*LG4 DNA (1 μg/ml) at a concentration of 2.5 μg of fragment DNA per ml. Transfections were performed in triplicate as described previously (13), and cultures were incubated at 34°C. Cell lysates were harvested 6 days after transfection and assayed on RSF cells at 34 and 39°C. The DNA fragments used were as follows: 1, 1,200-bp *Bam*HI-*Sal*I; 2, 516-bp *Sal*I-*Bal*I; 3, 1,225-bp *Sal*I-*Sst*I; 4, 628-bp *Nae*I-*Nae*I; 5, 858-bp *Sst*II-*Sst*I; 6, 792-bp *Stu*I-*Sst*I; and 7, 709-bp *Bal*I-*Sst*I. The percent of rescue (calculated as [virus titer at 39°C/virus titer at 34°C] × 100) is shown above each fragment. The ICP27 gene from mutant *ts*LG4 was cloned as a *Bam*HI-to-*Sst*I fragment in pUC18 (17). The region from nucleotides 1650 to 1950 within which the *ts* lesion was shown to map by the marker rescue analysis was sequenced along with the corresponding region from pSG130B/S. The sequence from nucleotides 1841 to 1936 is shown. In two independently cloned LG4 plasmids, there was a single base change, a transition from G to A, compared with the KOS sequence at nucleotide 1851. This results in a change from an arginine to a histidine residue two amino acids away from a putative zinc finger metal-binding domain of ICP27, shown by the boxed residues.

34°C. While there was a 14-fold reduction in viral yield at the nonpermissive temperature, there was a higher level of DNA replication seen 12 h after infection compared with the control level at 34°C (Fig. 7). This result is consistent with the phenotype of the viral mutant *ts*LG4, from which this cell line was derived. DNA synthesis in *ts*LG4-infected cells is nearly equivalent to the wild-type level at the nonpermissive temperature, yet late gene expression is severely reduced (36).

The results from the analysis of DNA replication and from the analysis of HSV-1 gene products by Northern blot and immunoprecipitation show that the ICP27 dominant activator mutants can interfere with the activation function of wild-type ICP27 during viral infection.

Mapping and sequencing of the LG4 mutation. The exact positions of the dominant insertion mutations are known, and they map to the activator domain between amino acids 262 and 434 (17). However, the site of the LG4 mutation was not known since the LG4 plasmid gene was cloned from the viral mutant *ts*LG4. The site of the *ts* lesion in this mutant was shown to map only to the ICP27 gene by marker rescue and complementation analysis (13, 36). Therefore, we performed a marker rescue experiment using a series of fragments within the ICP27 gene derived from the wild-type plasmid pSG130B/S (Fig. 8). Each of these fragments was cotransfected with DNA isolated from *ts*LG4. Transfected cultures were harvested 6 days later, and virus was assayed at both 34 and 39°C. The site of the mutation was shown to map between a *Bal*I site at nucleotide 1712 in the *Bam*HI-to-*Sst*I fragment of pSG130B/S and a *Nae*I site at 2012 on basis of the percent rescue obtained with fragments spanning this region and on the observation that the *Sal*I-*Bal*I fragment did not give rise to wild-type recombinants (Fig. 8). The coding region of ICP27 terminates at nucleotide 1949, so it was concluded that the mutation mapped between nucleotides 1712 and 1949, which defines what we have termed the repressor region of the ICP27 gene (17). To further locate the actual mutation, the region between nucleotides 1650 and 1950 was sequenced in two independently derived LG4 clones and in plasmid pSG130B/S. As shown in Fig. 8, a transition from G to A was found at nucleotide position 1851 in LG4. This results in the substitution of a histidine residue for an arginine at amino acid 480 from the predicted wild-type sequence (25). This substitution occurred within the repressor domain of ICP27 two residues away from the putative zinc finger motif (2, 3, 17, 31). However, the phenotype of LG4 is not that of a repressor mutant because it is not defective in the repressor function at the nonpermissive temperature (17), and it is dominant to wild type. The other mutants in the repressor domain are defective in both the activator and repressor functions (17), and these mutants were not found to be dominant (Table 1 and Fig. 2). One explanation for this difference is that the other repressor mutants contain insertions of three to five amino acids, whereas there was a single amino acid substitution in LG4. It is likely that this substitution alters the secondary structure of this important region to a lesser extent than occurs with the insertion mutants.

Because mutant LG4 was dominant in the transfection experiments with pVP5-CAT described earlier, and cell line

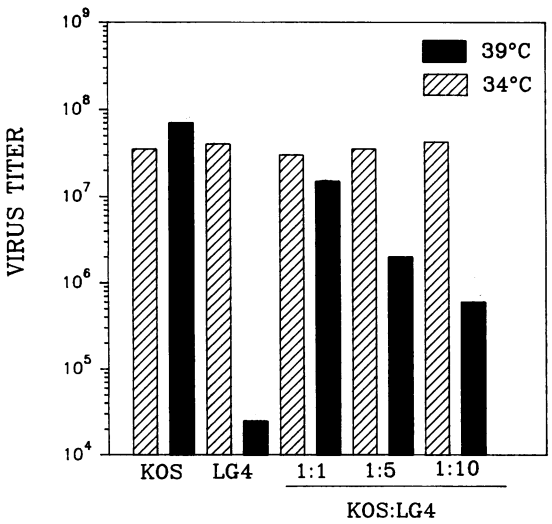

FIG. 9. Dominance of the *ts* mutation during coinfection of cells with wild-type HSV-1 KOS and *ts*LG4. RSF cells were infected with KOS or *ts*LG4 at a multiplicity of infection of 1 at either 34 or 39°C. Coinfection experiments were performed with KOS at a multiplicity of infection of 1 and *ts*LG4 at a multiplicity of 1, 5, or 10, as indicated, at 34 and 39°C. Infected cultures were incubated for 24 h, at which time virus yields were assayed on RSF cells at 34°C.

23-5 containing LG4 inhibited HSV-1 growth in a temperature-sensitive fashion, we tested the viral mutant *ts*LG4 in coinfection experiments with wild-type KOS. The infections were performed at different ratios of wild-type to mutant virus (Fig. 9). Infections were performed in RSF cells at 34 and 39°C, and virus yields were assayed at 34°C. The yield of infectious virus was about 100-fold lower when *ts*LG4 was added at a 10:1 ratio to KOS at 39°C than with the infections with KOS alone (Fig. 9). There was less of an inhibitory effect at ratios of 5:1 (35-fold) and 1:1 (3-fold), which is again consistent with the hypothesis that the mutant ICP27 protein competes with the wild-type protein.

DISCUSSION

The phenomenon of interference by viruses which contain mutations that behave in a dominant manner and can inhibit the growth of the wild-type virus in mixed infections has long been known for many viruses (for a review, see reference 46). As proposed by Herskowitz (18), the study of dominant mutations in regulatory proteins has led to the elucidation of some of the molecular interactions which these multifunctional polypeptides undergo (12, 23, 27, 29, 41). In this study, we tested a number of insertion mutants in the HSV-1 regulatory protein ICP27 for their ability to interfere with the activities of the wild-type protein. We found that mutants which were defective in the activator function but not the repressor activity were able to hinder the activation function of wild-type ICP27 in cotransfection experiments and were able to impair growth of HSV-1 KOS in cell lines which stably carried these mutant alleles. The defect in HSV-1 replication was found to be the result of a decreased expression of HSV-1 late gene products consistent with the activation role of ICP27 during infection.

In an earlier report, we showed that mutants in the carboxy-terminal half of ICP27 were defective in the ability to augment expression of an HSV-1 late target gene in transfection assays (17). However, mutants mapping between amino acids 260 and 434 of the 512-amino-acid protein were able to repress expression of an early target gene as efficiently as the wild-type plasmid. Therefore, we defined the activator region as the sequences between residues 260 and 434 and the repressor region as the terminal 78 amino acids of the protein, even though the repressor mutants are defective in both regulatory functions. Studies by Rice et al. (35) and McMahan and Schaffer (26) of ICP27 nonsense mutants which result in truncated polypeptides largely agree with the placement of these domains, except that McMahan and Schaffer (26) also defined a second weaker repressor region to lie between amino acids 327 and 407. The difference between our study and theirs was the analysis of full-length mutated proteins versus truncated polypeptides, so that differences in peptide folding would be expected to occur. In fact, we found an inconsistency in our own data in that LG4 maps to the repressor region but is not defective in that function and is dominant, unlike the other repressor mutants (Fig. 7). It was hypothesized that a single amino acid substitution would have a different effect on the folding of the peptide compared with the insertion of three to five amino acids in the other repressor mutants.

Analysis of viral mutants containing deletions and truncations in the ICP27 gene have disclosed a multitude of effects of ICP27 on viral gene expression, including a down regulation of immediate-early and early gene expression late in infection, an autoregulatory effect on its own expression, stimulation of leaky late gene products, induction of true late gene products, stimulation of viral DNA replication, and a modification in the mobility of ICP4 on sodium dodecyl sulfate-polyacrylamide gels (24, 26, 34). It is possible that some of these effects may be indirect, as a result of the inappropriate expression of some other viral gene product when ICP27 is defective, but even so, it is clear that ICP27 is a complex regulatory protein. We have chosen to concentrate in this study on the analysis of mutants which interfere with the wild-type activation function of ICP27 seen in transfection experiments and with the ability of the wild-type protein to activate late gene expression during viral infection. We did not find any mutants to be dominant for the repressor activities of ICP27 either in transfection experiments or during infection, nor did the dominant mutants interfere with viral DNA replication to a significant extent.

With the new evidence that the mutants within the activator region were dominant to the wild-type protein, we propose that ICP27 interactions occur through at least two functional domains, an activator domain and a binding region or interaction domain. While both regions of the ICP27 molecule probably interact with the substrate to carry out its function, we are suggesting that a binding region is required to initiate the interaction, so that if this domain is altered, binding to the substrate cannot occur. While some overlap probably occurs between the two regions which we have defined, because at least one activator mutant (LG4) maps to the repressor region (Fig. 8) and a weaker repressor region within the activator region has been found by McMahan and Schaffer (26), the binding domain could correspond to the repressor region of the molecule. In this way, an activator mutant of ICP27 having an intact repressor region could bind to its substrate, either another protein or nucleic acid; however, the binding would be nonfunctional because of the activator mutation. The mutant protein could interfere or compete with the wild-type binding to the substrate, resulting in the *trans*-dominance effect which we observed. Our data on the gene dosage of the mutants shown in Fig. 2

and 9 support this hypothesis. As shown in Table 1 and Fig. 2, mutants in the repressor region cannot interfere with either the repressor or activator function of the wild-type protein. This finding supports the notion that the repressor region is necessary for binding because mutations here would result in mutant polypeptides unable to bind the substrate and therefore unable to compete with the wild-type molecule. Further support for the proposal that the repressor region is involved in binding comes from the predicted amino acid sequence of the protein (25, 31). As we previously noted (17), there is a putative zinc finger metal-binding domain within this region (Fig. 8). We have evidence that the ICP27 protein binds zinc in vitro (45a). Given that zinc finger motifs have been shown to be involved in binding DNA and RNA and in protein-protein interactions (2, 3, 8, 11, 14, 15, 45), the presence of such a motif in the repressor region supports our hypothesis that this region is involved in binding. Clearly, definitive biochemical evidence, from studies in which both the substrate and the binding region are defined, is needed to support this claim. We have shown that ICP27 can bind to a DNA-agarose column containing double-stranded calf thymus DNA (45a), but we have not been able to demonstrate any sequence-specific binding. Recent evidence from our laboratory has shown that ICP27 acts, at least in part, posttranscriptionally to regulate gene expression both in transfection experiments (38a) and during viral infection (41a). Therefore, it may be more likely that ICP27 interacts directly with another protein or with RNA.

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REFERENCES

1. **Ackerman, M., D. K. Braun, L. Pereira, and B. Roizman.** 1984. Characterization of herpes simplex virus 1 alpha proteins 0, 4, and 27 with monoclonal antibodies. J. Virol. **52**:108–118.
2. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232**:485–487.
3. **Berg, J. M.** 1988. Proposed structure for the zinc-binding domains from transcription factor IIIa and related proteins. Proc. Natl. Acad. Sci. USA **85**:99–102.
4. **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.
5. **Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate early transcription. J. Mol. Biol. **180**:1–19.
6. **Cathala, G., J. F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial, and J. D. Baxter.** 1983. A method for the isolation of intact, translationally active ribonucleic acid. DNA **2**:329–335.
7. **DeLuca, N. A., A. M. McCarthy, and P. A. Schaffer.** 1985. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate-early regulatory protein ICP4. J. Virol. **56**:558–570.
8. **Evans, R. M., and S. M. Hollenberg.** 1988. Zinc fingers: gilt by association. Cell **52**:1–3.
9. **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.
10. **Fenwick, M. L., M. Walker, and L. Marshall.** 1978. On the association of virus proteins with the nuclei of cells infected with herpes simplex virus. J. Gen. Virol. **39**:519–529.
11. **Freedman, L. P., B. F. Luisi, Z. R. Korsun, R. Basavappa, P. B. Sigler, and K. R. Yamamoto.** 1988. The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. Nature (London) **334**:543–545.
12. **Friedman, A. D., S. J. Triezenberg, and S. L. McKnight.** 1988. Expression of a truncated viral *trans*-activator selectively impedes lytic infection by its cognate virus. Nature (London) **335**:452–454.
13. **Goldin, A. L., R. M. Sandri-Goldin, M. Levine, and J. C. Glorioso.** 1981. Cloning of herpes simplex type 1 sequences representing the whole genome. J. Virol. **38**:50–58.
14. **Green, L. M., and J. M. Berg.** 1989. A retroviral Cys-Xaa_2-Cys-Xaa_4-His-Xaa_4-Cys peptide binds metal ions: spectroscopic studies and a proposed three-dimensional structure. Proc. Natl. Acad. Sci. USA **86**:4047–4051.
15. **Green, S., V. Kumar, I. Theulaz, W. Wahli, and P. Chambon.** 1988. The N-terminal DNA-binding 'zinc finger' of the oestrogen and glucocorticoid receptors determines target specificity. EMBO J. **7**:3037–3044.
16. **Hanahan, D.** 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166**:557–580.
17. **Hardwicke, M. A., P. J. Vaughan, R. E. Sekulovich, R. O'Conner, and R. M. Sandri-Goldin.** 1989. The regions important for the activator and repressor functions of the HSV-1 alpha protein ICP27 map to the C-terminal half of the molecule. J. Virol. **63**:4590–4602.
18. **Herskowitz, I.** 1987. Functional inactivation of genes by dominant negative mutants. Nature (London) **329**:219–222.
19. **Holland, T. C., S. D. Marlin, M. Levine, and J. C. Glorioso.** 1983. Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. **45**:672–682.
20. **Knipe, D. M., and J. L. Smith.** 1986. A mutant herpesvirus protein leads to a block in nuclear localization of other viral proteins. Mol. Cell. Biol. **6**:2371–2381.
21. **Mackem, S., and B. Roizman.** 1982. Structural features of the herpes simplex virus alpha gene 4, 0, and 27 promoter-regulatory sequences which confer alpha regulation on chimeric thymidine kinase genes. J. Virol. **44**:939–949.
22. **Mackem, S., and B. Roizman.** 1982. Regulation of alpha genes of herpes simplex virus: the alpha 27 gene promoter-thymidine chimera is positively regulated in converted L cells. J. Virol. **43**:1015–1023.
23. **Malim, M. H., S. Bohnlein, J. Hauber, and B. R. Cullen.** 1989. Functional dissection of the HIV-1 rev *trans*-activator: derivation of a *trans*-dominant repressor of rev function. Cell **58**:205–214.
24. **McCarthy, A. M., L. McMahan, and P. A. Schaffer.** 1989. Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. J. Virol. **63**:18–27.
25. **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.
26. **McMahan, L., and P. A. Schaffer.** 1990. The repressing and enhancing functions of the herpes simplex virus regulatory protein ICP27 map to the C-terminal regions and are required to modulate viral gene expression very early in infection. J. Virol. **64**:3471–3485.
27. **Mermer, B., B. K. Felber, M. Campbell, and G. N. Pavlakis.** 1990. Identification of trans-dominant HIV rev protein mutants by direct transfer of bacterially produced proteins into human cells. Nucleic Acids Res. **18**:2037–2044.
28. **Neumann, J. R., C. A. Morency, and K. O. Russian.** 1987. A novel rapid assay for chloramphenicol acetyltransferase gene expression. Biotechniques **5**:444–447.

29. **Pearson, L., J. Garcia, F. Wu, N. Modesti, J. Nelson, and R. Gaynor.** 1990. A transdominant tat mutant that inhibits tat-induced gene expression from the human immunodeficiency virus long terminal repeat. Proc. Natl. Acad. Sci. USA **87**:5079–5083.
30. **Pereira, L., M. H. Wolff, M. Fenwick, and B. Roizman.** 1977. Regulation of herpesvirus macromolecular synthesis. V. Properties of alpha polypeptides made in HSV-1 and HSV-2 infected cells. Virology **77**:733–749.
31. **Perry, L. J., and D. J. McGeoch.** 1988. The DNA sequences of the long repeat region and adjoining parts of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69**:2831–2846.
32. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of alpha genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. Cell **24**:555–565.
33. **Rice, S. A., and D. M. Knipe.** 1988. Gene-specific transactivation by herpes simplex virus type 1 alpha protein ICP27. J. Virol. **62**:3814–3823.
34. **Rice, S. A., and D. M. Knipe.** 1990. Genetic evidence for two distinct transactivation functions of the herpes simplex virus alpha protein ICP27. J. Virol. **64**:1704–1715.
35. **Rice, S. A., L. Su, and D. M. Knipe.** 1989. Herpes simplex virus alpha protein ICP27 possesses separable positive and negative regulatory activities. J. Virol. **63**:3399–3407.
36. **Sacks, W. R., C. C. Greene, D. P. Ashman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.
37. **Sandri-Goldin, R. M., A. L. Goldin, L. E. Holland, J. C. Glorioso, and M. Levine.** 1983. Expression of herpes simplex virus beta and gamma genes integrated in mammalian cells and their induction by an alpha gene product. Mol. Cell. Biol. **3**:2028–2044.
38. **Sandri-Goldin, R. M., M. Levine, and J. C. Glorioso.** 1981. Method for induction of mutations in physically defined regions of the herpes simples virus genome. J. Virol. **38**:41–49.

38a. **Sandri-Goldin, R. M., and G. E. Mendoza.** Submitted for publication.

39. **Sandri-Goldin, R. M., R. E. Sekulovich, and K. Leary.** 1987. The alpha protein ICP0 does not appear to play a major role in the regulation of herpes simplex virus gene expression during infection in tissue culture. Nucleic Acids Res. **15**:905–919.
40. **Sekulovich, R. E., K. Leary, and R. M. Sandri-Goldin.** 1988. The herpes simplex virus type 1 alpha protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP4 and ICP0. J. Virol. **62**:4510–4522.
41. **Shepard, A. A., P. Tolentino, and N. A. DeLuca.** 1990. *trans*-dominant inhibition of herpes simplex virus transcriptional regulatory protein ICP4 by heterodimer formation. J. Virol. **64**:3916–3926.

41a. **Smith, I. L., M. A. Hardwicke, and R. M. Sandri-Goldin.** Submitted for publication.

42. **Smith, I. L., and R. M. Sandri-Goldin.** 1988. Evidence that transcriptional control is the major mechanism of regulation for the glycoprotein D gene in herpes simplex virus type 1-infected cells. J. Virol. **62**:1474–1477.
43. **Su, L., and D. M. Knipe.** 1989. Herpes simplex virus alpha protein ICP27 can inhibit or augment viral gene transactivation. Virology **170**:496–504.
44. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
45. **Umensono, K., and R. M. Evans.** 1989. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell **57**:1139–1146.

45a. **Vaughan, P. J., K. J. Thibault, and R. M. Sandri-Goldin.** Submitted for publication.

46. **Whitaker-Dowling, P., and J. S. Youngner.** 1987. Viral interference-dominance of mutant viruses over wild-type virus in mixed infections. Microbiol. Rev. **51**:179–191.
47. **Whitton, J. L., F. J. Rixon, A. J. Easton, and J. B. Clements.** 1983. Immediate-early mRNA-2 of herpes simplex viruses type 1 and type 2 is unspliced: conserved sequences around the 5′ and 3′ termini correspond to transcription regulatory signals. Nucleic Acids Res. **11**:6271–6287.
48. **Zweig, M., C. R. Heilman, H. Rabin, R. F. Hopkins, R. H. Neubauer, and B. Hampar.** 1979. Production of monoclonal antibodies against nucleocapsid proteins of herpes simplex virus types 1 and 2. J. Virol. **32**:676–678.