Gene-Specific Transactivation by Herpes Simplex Virus Type 1 Alpha Protein ICP27

STEPHEN A. RICE AND DAVID M. KNIPE*

Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115

Received 6 May 1988/Accepted 9 July 1988

Herpes simplex virus type 1 (HSV-1) encodes several alpha (immediate-early) gene products that modulate gene expression during viral replication. We report here that the alpha protein ICP27 specifically stimulates expression of a later viral gene, that encoding glycoprotein B (gB). Using temperature-sensitive viral mutants, the effect of ICP27 on HSV-1 protein synthesis was examined at early times after infection or at later times when viral DNA replication was inhibited. Under these conditions, the expression of gB showed a marked dependence on the presence of functional ICP27, whereas several other beta and gamma 1 genes showed a lesser dependence. It was also noted that cells infected with ICP27 temperature sensitive mutants at the nonpermissive temperature showed a reduction in the electrophoretic mobility of the alpha protein ICP4. To examine the mechanism by which ICP27 stimulated gB expression, a plasmid was constructed in which the promoter-regulatory region of the gB gene was fused to the gene encoding chloramphenicol acetyltransferase (CAT). CAT expression from this plasmid was induced significantly by ICP27 expressed from a cotransfected plasmid. Induction of CAT activity by ICP27 correlated well with an increase in the amount of CAT transcripts initiated from the transcriptional start site of the gB gene. The transactivating activity of ICP27 was specific for the gB promoter-regulatory region, as expression from several other HSV-1 promoter-CAT chimeric genes was not stimulated by ICP27. The DNA sequences which conferred the response to ICP27 mapped within 175 base pairs upstream and 41 base pairs downstream of the gB transcriptional start site. Our results suggest that the full expression of gB and perhaps other viral genes during HSV-1 infection requires the combined action of multiple viral transactivators.

Eucaryotic cells and their viruses encode proteins which regulate gene expression and show various levels of specificity for target genes (31, 34). Herpes simplex virus type 1 (HSV-1) encodes several alpha (immediate-early) gene products that regulate viral gene expression during the lytic cycle of viral replication. To date, the action of these gene products has been shown to be general for the stimulation of HSV-1 gene expression. We report here on the specific stimulation of an HSV-1 gene by the alpha protein ICP27.

The expression of viral genes during HSV-1 infection occurs in a tightly regulated cascade pattern (5, 23, 25; for a review, see reference 47). HSV-1 genes have been classified into several groups based on the temporal order and functional requirements for their expression. The first set of viral genes to be expressed are the alpha or immediate-early genes. The transcription of alpha genes does not require synthesis of any new viral proteins (23) but is enhanced by a component of the incoming virus particle (4, 41). The next set of genes to be transcribed is the beta, or delayed-early, genes. Many of the beta proteins are required for viral DNA replication. The gamma or late genes are the last temporal class of genes to be activated, and their full expression is dependent upon the commencement of viral DNA synthesis (20, 23). Gamma genes have been divided into two groups: gamma 1 genes, the transcripts of which are expressed at low levels even in the absence of DNA synthesis, and gamma 2 genes, the transcripts of which are expressed at undetectable or barely detectable levels in the absence of viral DNA replication.

Expression of functional alpha proteins is required for the activation of the beta and gamma classes of HSV genes (24).

Five alpha proteins have been identified: infected-cell polypeptides (ICPs) 0, 4, 22, 27, and 47. The genes specifying two of these polypeptides, ICP22 and ICP47, can be deleted from the virus without adverse effects on viral growth in most cell lines (32, 42). The three remaining alpha proteins, ICP4, ICP0, and ICP27, are nuclear phosphoproteins with apparent molecular weights of approximately 165,000, 110,000, and 63,000, respectively (1, 23, 27, 37, 40, 44). All three proteins have been implicated to some extent in the activation of HSV-1 beta and gamma gene expression. Evidence for this comes from the results of two types of studies. The first is analysis of viral mutants containing deletions or temperature-sensitive (*ts*) mutations in the genes specifying these proteins. The second is transient expression analysis in which beta or gamma HSV-1 genes or promoters are transfected into uninfected cells together with genes specifying alpha proteins.

Evidence from both types of studies has implicated ICP4 as an important activator of beta and gamma gene transcription. At the nonpermissive temperature, viral mutants containing *ts* mutations in the ICP4 gene fail to accumulate or transcribe beta and gamma mRNAs (15, 43, 54). Viruses containing deletions in the ICP4 gene also fail to induce beta and gamma genes and are completely nonviable in normal cell lines (8). Transient expression analyses also implicate ICP4 as an inducer of gene expression, because many beta and gamma genes and promoters introduced into cells by transfection can be transactivated by ICP4 (9, 10, 12, 33, 38, 39, 45). Partially purified preparations of ICP4 have been shown to bind to DNA in a sequence-specific manner (11). However, since many HSV genes activated by ICP4 do not appear to contain ICP4-binding sites, it is unclear whether

* Corresponding author.

this apparent biochemical activity of ICP4 is relevant to its ability to induce gene expression.

Transient assay experiments also suggest that ICP0 may be involved in the stimulation of beta and gamma gene expression. Many HSV genes or promoters are activated by cotransfection with plasmids encoding ICP0 (10, 12, 33, 38, 39, 45). In some cases, ICP0 appears to work synergistically with ICP4 in transactivating HSV promoters (38, 45). HSV-1 mutants which contain deletions in the ICP0 gene have recently been isolated (50, 53). These mutants are impaired for growth, especially at low multiplicities of infection, but are viable. Surprisingly, these mutants do not exhibit any dramatic defects in viral gene expression. Thus, although ICP0 can be a potent activator of gene expression in transfected cells, its role in the activation of beta and gamma gene expression during lytic infection is presently unclear.

The alpha protein ICP27 has been suggested to play an essential role in HSV-1 gene activation because viral mutants containing *ts* lesions in the ICP27 gene fail to accumulate gamma 2 and, to a lesser extent, beta and gamma 1 proteins at the nonpermissive temperature (49). These mutants are able to replicate their DNA at the nonpermissive temperature (49, 51). In transient assay experiments, ICP27 has generally failed to affect expression of cotransfected HSV genes (9, 33, 38). Everett, however, recently reported (10) that the promoter of the ICP5 (gamma 1) gene can be stimulated in a transient expression assay by the combined effect of ICP27, ICP4, and ICP0, although ICP27 alone has no effect.

The present studies were initiated to further explore the role ICP27 plays in activating HSV-1 gene expression. We find that viral mutants containing *ts* lesions in the ICP27 gene show a markedly reduced ability to express the viral glycoprotein B (gB) gene, whereas expression of other beta and gamma 1 genes is affected to a lesser extent. In addition, we demonstrate that the promoter-regulatory region of the gB gene can be specifically transactivated in transfected cells by the action of ICP27.

MATERIALS AND METHODS

Cells, viruses, and infections. All infections and transfections were carried out in Vero cells propagated from a stock originally obtained from the American Type Culture Collection, Rockville, Md. HSV-1 KOS1.1 was originally provided by M. Levine (University of Michigan, Ann Arbor). Strain KOS1.1 *ts*LG4 (51) was obtained from R. Sandri-Goldin (University of California-Irvine), and KOS *ts*Y46 (49) was provided by P. Schaffer (Dana-Farber Cancer Institute, Boston, Mass.).

A *ts*⁺ derivative of *ts*LG4 was obtained in the following way. Purified *ts*LG4 DNA (1.5 µg) was mixed with 30 molar-equivalents of pBH27 DNA which had been digested with *Bam*HI and *Hind*III to release the viral DNA insert from the vector sequences. The DNA was transfected into Vero cells, and progeny virus was harvested at 4 days after transfection. The titers of the progeny were determined at the permissive and nonpermissive temperatures to determine if rescue of the *ts* mutation had occurred. The plasmid pBH27 was able to rescue, but two control plasmids, pUC19 and pEK4, were not. One *ts*⁺ virus plaque isolate was obtained and was plaque purified two additional times. This virus was designated KOS1.1 LG4-rl.

Infections were carried out at a multiplicity of 20 PFU per cell at the nonpermissive temperature, 39.7°C, or at the permissive temperature, 34°C. Disodium phosphonacetate (a

gift from Abbott Laboratories, North Chicago, Ill.) was used at a concentration of 400 µg/ml.

Labeling of infected cells and gel electrophoresis. Infected cells were labeled for 20 min at 39.7°C or 40 min at 34°C with 20 µCi of [³⁵S]methionine per ml. Harvesting of infected cells and sodium dodecyl sulfate (SDS)-gel electrophoresis in 9.25% acrylamide gels were carried out as previously described (14, 28).

Recombinant plasmids. The plasmid pSG1-ES1, which contains the ICP0 gene, has been previously described (45). The plasmid pEK4, which contains the ICP4 gene, has the same 8.5-kilobase (kb) *Eco*RI-*Kpn*I HSV-1 insert as the previously described plasmid pSG1-EK1 (45) but with the insert recloned into pUC19. The plasmid pBH27, which contains the ICP27 gene, has a 2.4-kb HSV-1 KOS1.1 *Bam*HI-*Hpa*I DNA fragment cloned between the *Bam*HI and *Hinc*II sites of pUC19. A mutated version of pBH27, pBH27X1, was obtained from L. Su (L. Su and D. Knipe, manuscript in preparation). This plasmid is identical to pBH27 except for containing a synthetic linker oligonucleotide with stop codons in all three reading frames inserted into the *Sal*I site of the ICP27-coding region. The plasmids pVP5-CAT and pAE-CAT (6) were provided by E. Wagner (University of California-Irvine) and contain promoter sequences from nucleotides -650 to -10 and -200 to +30, relative to the transcriptional start site of the ICP5 and alkaline exonuclease genes, respectively. The plasmids pICP8-CAT and pTK-CAT were provided by L. Su and contain promoter sequences from nucleotides -335 to +93 and -650 to +50, relative to the transcriptional start sites of the ICP8 and thymidine kinase genes, respectively (L. Su and D. Knipe, manuscript in preparation).

The plasmid pgB-CAT was constructed in the following manner. The 545-base-pair (bp) *Xho*I fragment containing the gB promoter was isolated from pSG18 (16). This fragment contains sequences from nucleotides -504 to +41 relative to the approximate transcriptional start site of the gB gene. The transcriptional start site of the gB mRNA has been mapped by primer extension analysis to a site approximately 189 nucleotides upstream of an *Acc*I site in the gB gene (22) but has not been mapped to a specific nucleotide. For ease of discussion in this study, we are designating the first A residue in the sequence TCGCCACCACA (3), which is 24 nucleotides 3' to a TATA homology, as the initiation site, or nucleotide +1, of the gB promoter. The 3' recessed ends were filled in by using the Klenow fragment of *E. coli* DNA polymerase (30), and *Hind*III linkers (New England BioLabs, Beverly, Mass.) were ligated to the fragment. After digestion with *Hind*III, this DNA was inserted into the *Hind*III site of pSV0d (ori⁻)-CAT (6; provided by E. Wagner). A transformant carrying the gB promoter inserted correctly relative to the CAT gene was isolated.

The plasmid pgB-CAT-5'del(-175) and other 5' deletion derivatives were constructed in the following manner. The pgB-CAT plasmid was linearized by digestion with *Sac*II, which cleaves the HSV sequences 447 bp upstream of the gB transcriptional start site. Deletions were then generated by using BAL 31 nuclease (30). The DNA was then restricted with *Sal*I, which cleaves the vector sequence at a distal site. The DNA ends were repaired with the Klenow fragment of *E. coli* DNA polymerase, and an *Xba*I linker (New England BioLabs) was added. After restriction with *Xba*I and religation, the DNA was used to transform *E. coli*. Individual transformants were isolated, their structure was confirmed by restriction enzyme digestion, and the extents of the deletions were determined by DNA sequencing.

The plasmid pgB-CAT-s(-240) is a subclone of pgB-CAT in which a 1.1-kb *BclI-EcoRI* fragment was cloned into pUC19 between the *BamHI* and *EcoRI* sites. The 1.1-kb fragment contains the gB promoter sequences from -240 to +41 relative to the transcriptional start site, as well as the entire CAT gene, including the polyadenylation site.

All plasmid DNAs were purified by a cleared lysate procedure, followed by ethidium bromide-cesium chloride ultracentrifugation (30).

Transfections. Vero cells were transfected by using the calcium phosphate precipitation procedure (18). For CAT assays, transfections were done with 25-cm² culture flasks of Vero cells by using 8 µg of CAT plasmid and 4 µg of each plasmid containing HSV alpha genes. Carrier pUC19 DNA was added to bring the total amount of DNA in each transfection to 16 µg. For preparation of RNA, 150-cm² flasks were transfected with 50 µg of CAT plasmid, 25 µg of pBH27, and enough pUC19 DNA to bring the total amount to 100 µg. The calcium phosphate-DNA precipitates were allowed to sit for 8 to 15 min and then added to the flasks. After 20 to 30 min, medium containing 2% fetal calf serum was added to each flask. The transfected cells were incubated at 37°C for 4 to 5 h. The medium was then changed to medium containing 6% fetal calf serum. The transfections were incubated for 40 to 50 h at 37°C, and then cells were harvested.

CAT assays. Chloramphenicol acetyltransferase (CAT) activity in transfected cells was determined in a standard assay (17). One half of the extract from cells of each culture was assayed in a total volume of 150 µl containing 150 mM Tris hydrochloride, pH 7.8, 0.5 mM acetyl coenzyme A (Pharmacia, Inc., Piscataway, N.J.), and 0.3 µCi of [¹⁴C]chloramphenicol for 1 h at 37°C. After thin-layer chromatography, the individual spots corresponding to the substrate and products were identified by autoradiography and cut out, and their radioactivity was determined by liquid scintillation counting. In each experiment, 16 µg of pUC19 DNA was transfected as a control, and the percent acetylation obtained in this case (usually about 0.15%) was considered background and subtracted from each value. Control experiments showed that the assay was linear with respect to CAT activity up to at least 40% acetylation of the substrate. When 40% acetylation was exceeded, 5- or 25-fold dilutions of the sample were made, and the assay was repeated to give a value below 40% acetylation. This value was then multiplied by the dilution used to determine the relative CAT activity (6).

RNase protection assays. Cytoplasmic RNA was prepared from transfected cells by phenol and chloroform extraction of the 0.5% Nonidet P-40 supernatant fractions, followed by ethanol precipitation (26). The RNA was then treated with RNase-free DNase I (Bethesda Research Laboratories, Gaithersburg, Md.) to digest any residual plasmid DNA.

An SP6 antisense RNA expression vector was constructed by subcloning a *PvuII-XbaI* fragment from a gB-CAT 5' deletion derivative, pgB-CAT-5'del(-104) into the *XbaI* and *SmaI* sites of pSP65 (Promega Biotec, Madison, Wis.). This plasmid, pRP2, was linearized by digestion with *XbaI* prior to in vitro transcription. The ³²P-labeled probe was prepared and the RNase protection assays were performed essentially as described by Zinn et al. (59). Equal amounts of cytoplasmic RNA, usually 50 µg, were used to protect 10⁶ cpm of ³²P-labeled antisense probe. The protected fragments were analyzed on 6% denaturing urea-acrylamide gels, followed by autoradiography.

RESULTS

HSV ICP27 *ts* mutants exhibiting defects in gB expression. Sacks et al. (49) previously used long-term protein labeling experiments to demonstrate that HSV-1 *ts* mutants with mutations in the ICP27 gene are defective at the nonpermissive temperature for the accumulation of gamma 2 proteins and, to a lesser degree, beta and gamma 1 proteins. Although these mutants replicate viral DNA at the nonpermissive temperature (49, 51), we observed that viral DNA replication was substantially reduced in ICP27 mutant-infected cells (data not shown). Because full expression of gamma genes is dependent upon viral DNA replication, it was possible that some of the defects in gene expression previously reported for the ICP27 *ts* mutants were due to a reduced rate of DNA replication and not to a direct effect of ICP27 on viral gene expression. To address this point, we examined protein synthesis in cells at early times after infection, before the onset of DNA replication, or under conditions in which DNA replication was blocked by a specific inhibitor of HSV DNA synthesis, phosphonoacetic acid. This allowed us to compare the ability of wild-type and mutant viruses to express their genes independent of their ability to replicate viral DNA.

Vero cells were infected with wild-type HSV-1 KOS1.1 or the ICP27 *ts* mutant KOS *ts*LG4 (51), and at various times after infection, the cells were pulse-labeled with [³⁵S]methionine. Protein synthesis was analyzed by SDS-gel electrophoresis and autoradiography (Fig. 1). At 3 h after infection, both wild-type- and mutant-infected cells showed a very similar pattern of protein synthesis at the nonpermissive temperature, although two major quantitative differences were observed (Fig. 1, lanes 3 and 4). First, consistent with previous observations (49), the ICP27 mutant overexpressed the alpha protein ICP4. Second, the synthesis of the precursor to gB (pgB; 52) was markedly reduced in the mutant-infected cells. At 5 and 7 h postinfection, many qualitative differences between wild-type- and mutant-infected cell protein synthesis became apparent (Fig. 1, lanes 5 and 6 and lanes 7 and 8). Because DNA synthesis begins between 3 and 6 h postinfection (48, 55), some of the differences seen at these times may be due to a reduction in DNA replication in *ts*LG4-infected cells. Indeed, there were fewer differences at 7 h postinfection when viral DNA replication was inhibited, but significant differences remained (Fig. 1, lanes 9 and 10). Although the most striking difference under these conditions was a reduction in the amount of pgB synthesized in *ts*LG4-infected cells, other proteins, such as ICP8, were also underexpressed. In contrast, the gamma 1 protein ICP5 was reduced only marginally if at all. Thus, it appears that HSV-1 late proteins (beta and gamma 1) were differentially affected by *ts* mutations in ICP27. It is also apparent that the alpha protein ICP4 was overexpressed in *ts*LG4-infected cells in the presence of phosphonoacetic acid.

Additional experiments were performed to correlate *ts* defects in ICP27 with the inability to fully activate gB expression at early times after infection. First, we examined gene expression in cells infected with KOS LG4-rl, a *ts*⁺ virus obtained by marker rescue of *ts*LG4 with a cloned, wild-type copy of the ICP27 gene. Synthesis of pgB at 3 h postinfection was quite reduced at the nonpermissive temperature in *ts*LG4-infected cells but occurred normally in cells infected with *ts*LG4-rl (Fig. 2, lanes 3 and 4). Thus, the inability of *ts*LG4 to fully activate pgB expression at early times after infection is due to a defect in its ICP27 gene. Second, we examined protein synthesis in cells infected with

FIG. 1. Protein synthesis in ICP27 mutant-infected cells. Vero cells were mock-infected or infected with KOS1.1 or KOS1.1 *ts*LG4 at a multiplicity of infection of 20. The cells were incubated at 39.7 or 34°C (lane 2). Some infections (lanes 9 and 10) were carried out in the presence of 400 μ g of phosphonacetic acid (PAA) per ml. At the indicated times, the cells were pulse-labeled for 20 min at 39.7°C or 40 min at 34°C with [35 S]methionine. Equal fractions of each cell lysate were subjected to SDS-gel electrophoresis and autoradiography. Lane 11 contains 14 C-labeled protein molecular size standards (MW stds.). The positions of several HSV-1 proteins are indicated at the left of the gel, and the molecular sizes of the 14 C-labeled standards are indicated at the right (in kilodaltons [kd]).

another ICP27 *ts* mutant, KOS *ts*Y46 (49). Cells infected with *ts*Y46 also showed low levels of pgB expression at the nonpermissive temperature (Fig. 2, lane 5). Therefore, the inability to fully activate gB expression at early times after infection is a property shared by two independent ICP27 *ts* mutants. The *ts* mutants also failed to synthesize normal levels of some other beta or gamma 1 proteins, such as ICP8, although at least one gamma 1 protein, ICP5, was reduced only marginally in the *ts* mutant-infected cells.

The experiment shown in Fig. 2 demonstrates two additional points concerning the phenotype of the ICP27 *ts* mutants. First, in addition to ICP4, the alpha protein ICP0 was also expressed at a higher than normal rate at the nonpermissive temperature in the *ts* mutant-infected cells. Second, the *ts* mutant-infected cells not only overexpressed the ICP4 protein at the nonpermissive temperature but also synthesized an ICP4 with an altered electrophoretic mobility. The ICP4 synthesized during a pulse-label in the mutant-infected cells migrated as a more diffuse band than did the wild-type ICP4 and showed a reduced electrophoretic mobility.

FIG. 2. Protein synthesis in ICP27 *ts* mutant-infected cells. Vero cells were mock-infected or infected at a multiplicity of infection of 20 with KOS1.1, LG4r1, *ts*LG4, or *ts*Y46 and incubated at 39.7 or 34°C. At 3 h after infection, the cells were pulse-labeled for 20 min at 39.7°C or 40 min at 34°C with [35 S]methionine. Equal fractions of each cell lysate were subjected to SDS-gel electrophoresis and autoradiography. Shown at the right of the figure are the positions of several HSV-1 proteins.

ICP27-stimulated expression of a gB-CAT chimeric gene.

The above experiments suggested that functional ICP27 is required for complete activation of gB expression during HSV-1 infection. Thus, it seemed possible that ICP27 stimulates expression of the gB gene introduced into cells by transfection. In pilot transfection experiments, we observed that a plasmid encoding ICP27 could enhance the ability of a plasmid encoding both ICP4 and ICP0 to stimulate expression of a third plasmid containing the intact gB gene (data not shown). To study this phenomenon in more detail, the promoter-regulatory region of the gB gene was fused to the structural gene encoding bacterial CAT (17). The resulting plasmid pgB-CAT contains a 545-bp HSV-1 DNA insert with sequences from nucleotides -504 to +41 relative to the gB transcriptional initiation site placed 5' to the CAT-coding region.

We examined the effect of cotransfection of various immediate-early gene plasmids (Fig. 3) on CAT expression from pgB-CAT (Fig. 4). In the absence of immediate-early genes, expression from pgB-CAT was barely detectable (Fig. 4, lane 1). Like many HSV promoter-regulatory regions, that of gB was stimulated by both ICP4 and ICP0 (lanes 2, 4, and 6). When pBH27, a plasmid encoding ICP27, was added in combination with plasmids encoding ICP4, ICP0, or both, greater stimulation of CAT expression was obtained compared with that without the ICP27 plasmid. Furthermore, the plasmid encoding ICP27 alone was able to transactivate pgB-CAT (lane 8). Transactivation of pgB-CAT by pBH27 was observed in numerous repeat experiments using different preparations of both plasmid DNAs.

To investigate the sequences in pgB-CAT required for its response to ICP27, two derivatives of pgB-CAT were constructed. One plasmid, pgB-CAT-5'del(-175), contained a deletion extending from bp -504 to -175 relative to the gB transcriptional start site. This plasmid was similar to the

FIG. 3. Structure of the HSV-1 genome and HSV-1 recombinant plasmids. (A) Schematic representation of the prototype arrangement of the HSV-1 genome. The boxed segments denote repeated sequences, while the dotted line indicates the joint between the long and short components of the genome. The arrows indicate the location and direction of transcription of the genes relevant to this study. Below are shown the map coordinates of the genome. (B) HSV-1 inserts of the recombinant plasmids used in this study. The arrows denote the primary transcripts encoding the indicated proteins. Relevant restriction sites are shown. E, *EcoRI*; K, *KpnI*; S, *SacI*; B, *BamHI*; H, *HpaI*.

parental construct in both its very low basal level of CAT activity and its response to transactivation by the ICP27 plasmid (data not shown). Another construct was engineered in which a portion of the gB promoter (nucleotides -240 to +41) as well as the entire CAT gene was subcloned into a new vector, pUC19. This plasmid, pgB-CAT-s(-240), showed a substantially higher level of basal CAT expression than did pgB-CAT (50- to 100-fold) but was still inducible by pBH27 (3- to 5-fold; data not shown). These results indicate that vector sequences could influence basal CAT expression in this system but they were not responsible for the response to pBH27. Furthermore, the sequences which were responsive to transactivation by the ICP27 plasmid mapped between nucleotides -175 and +41 relative to the gB transcriptional start site.

Requirement of functional ICP27 for transactivation. Stimulation of CAT activity from pgB-CAT and its derivatives by cotransfection with the ICP27 plasmid might be an effect mediated by the ICP27 protein. Alternatively, the ICP27 plasmid DNA itself could activate pgB-CAT expression by competing for *trans*-acting factors which repress gB-CAT expression. To distinguish between these possibilities, we compared the transactivation ability of the wild-type ICP27 plasmid pBH27 to that of a mutated version, pBHx1. The mutant plasmid is identical to pBH27 except for containing a 14-bp synthetic linker inserted at a *SacI* site in approximately the middle of the ICP27-coding region (L. Su and D. Knipe, unpublished results). The linker contains stop codons in all

FIG. 4. Stimulation of expression of pgB-CAT by plasmids encoding HSV-1 alpha proteins. Vero cells were transfected with 8 μ g of pgB-CAT plus the following DNAs: lane 1, 8 μ g of pUC19; lane 2, 4 μ g of pEK4; lane 3, 4 μ g each of pEK4 and pBH27; lane 4, 4 μ g of pSG1-ES1; lane 5, 4 μ g each of pSG1-ES1 and pBH27; lane 6, 4 μ g of pSG1; lane 7, 4 μ g each of pSG1 and pBH27; lane 8, 4 μ g of pBH27. Carrier pUC19 DNA was added to all transfections to give a total of 16 μ g. The transfected cells were harvested after 2 days, and one half of each cell lysate was assayed for CAT activity by using [14 C]chloramphenicol as a substrate. The reaction products and substrate were separated by thin-layer chromatography, and an autoradiogram of the thin-layer plate is shown. At the right of the figure are indicated the positions of the acetylated products (1Ac-C and 3Ac-C) and the substrate chloramphenicol (C). At the top of the figure, the alpha proteins expressed in each transfection are indicated. Also indicated is the fold stimulation of CAT expression from pgB-CAT by cotransfection with the plasmids encoding HSV-1 alpha proteins. The level of uninduced CAT expression from pgB-CAT in this experiment was 0.18% acetylation (lane 1).

three reading frames, and thus pBHx1 is predicted to encode a truncated form of ICP27.

Increasing amounts of wild-type or mutant ICP27 plasmid were added to a fixed amount of pgB-CAT-5'del(-175) (8 μ g). The DNA was transfected into cells, and relative CAT activities were determined after 2 days (Fig. 5). The wild-

FIG. 5. Transactivation requires a functional ICP27 polypeptide. Vero cells were transfected with 8 μ g of pgB-CAT-5'del(-175) plus increasing amounts of pBH27 (○) or pBHx1 (●). The cells were harvested after 2 days, and CAT activities were determined.

TABLE 1. Specificity of transactivation by ICP27

Plasmid transfected ^a	CAT activity ^b		
	Expt 1	Expt 2	Expt 3
pgB-CAT	0.15	0.20	ND
+ pEK4	1.27 (8.5)	2.40 (12)	ND
+ pBH27	0.70 (4.7)	1.27 (6.4)	ND
pICP8-CAT	11.1	7.03	9.70
+ pEK4	323 (29)	319 (45)	191 (20)
+ pBH27	4.54 (0.4)	5.06 (0.7)	3.08 (0.3)
pTK-CAT	0.73	0.56	0.98
+ pEK4	101 (139)	45.6 (81)	103 (105)
+ pBH27	1.32 (1.8)	0.74 (1.3)	0.80 (0.8)
pAE-CAT	0.18	0.16	<0.05
+ pEK4	0.97 (5.4)	1.31 (8.2)	0.45
+ pBH27	0.19 (1.1)	0.26 (1.6)	0.09
pICP5-CAT	<0.05	<0.05	<0.05
+ pEK4	0.13	0.05	0.05
+ pBH27	<0.05	<0.05	<0.05

^a CAT plasmid (8 µg) and 4 µg of pEK4 or pBH27 were transfected into a 25-cm² culture flask. The cells were harvested at 44 to 52 h posttransfection.

^b Protein extracts were assayed for CAT activity, and relative CAT activities were determined. The level of induction of each CAT construct (relative to the uninduced activity, defined as 1) is shown in parentheses. ND, Not done.

type plasmid was able to significantly transactivate pgB-CAT-5'del(-175) expression even at the lowest amount added (0.5 µg). The stimulation increased with increasing amounts of the pBH27 plasmid and appeared to plateau with approximately 4 µg of plasmid DNA. In contrast, the mutated pBH27 was inactive when added in small amounts but showed a small amount of stimulation when larger amounts were added. These results demonstrate that at least the majority of the transactivating ability of pBH27 is due to its capacity to encode an intact ICP27. It is unclear whether the remaining stimulation by pBH27 is due to residual activity of the truncated protein or to DNA competition effects.

Promoter-specific transactivation by ICP27. Several HSV-1 genes or promoter-test gene fusions are transactivated in transient expression assays by the action of ICP4 and ICP0 (9, 33, 38). These previous experiments have not demonstrated a similar function for ICP27. Everett, however, recently reported that an ICP5-CAT construct could be activated by an ICP27 plasmid but only when transfected in combination with plasmids encoding ICP4 and ICP0 (10). To determine whether transactivation by ICP27 in our system was specific for the gB promoter, we tested several HSV promoter-CAT fusions in cotransfection experiments. These constructs contained beta (thymidine kinase [TK], ICP8, and alkaline exonuclease [AE]) or gamma 1 (VP5 = ICP5) promoters. A series of experiments was performed in which CAT plasmids were transfected alone or were cotransfected with pBH27, which encodes ICP27, or pEK4, which encodes ICP4. Equal fractions of the transfected cell extracts were assayed for CAT activity, and the results of three independent transfection experiments are shown in Table 1.

The various CAT plasmids differed significantly in their uninduced levels of expression, with pICP8-CAT showing the most activity, followed by pTK-CAT. The plasmids pgB-CAT and pAE-CAT were only marginally active, while the pVP5-CAT construct was inactive. With the exception of pVP5-CAT, all of the constructs could be transactivated significantly by cotransfection with a plasmid expressing

FIG. 6. ICP27 stimulates the accumulation of correctly initiated gB-CAT transcripts in transfected cells. nt, Nucleotides. (A) Schematic representation of the strategy used to map gB-CAT transcripts by the RNase protection assay. At the right, the sizes of the full-length RNA probe and the major expected protected fragments are indicated. (B) RNase protection of gB-CAT transcripts. Vero cells were transfected as described in Materials and Methods with pUC19 DNA only (lane 2), pgB-CAT (lane 3), pgB-CAT plus pBH27 (lane 4), pgB-CAT-5'del(-175) (lane 5), pgB-CAT-5'del(-175) plus pBH27 (lane 6), pgB-CAT-s(-240) (lane 7), pgB-CAT-s(-240) plus pBH27 (lane 8), and pSV2-CAT (lane 9). Cytoplasmic RNA was prepared and annealed to the ³²P-labeled antisense probe. RNA (50 µg) was added in lanes 2 through 8; in lane 9, 10 µg of pSV2-CAT transfected cell RNA was added. Lane 1 contains ³²P-labeled DNA markers, the sizes of which are indicated at the left of the panel in nucleotides. At the right are indicated the sizes in nucleotides of the major protected RNA fragments.

ICP4, although the level of stimulation by ICP4 varied somewhat among the CAT plasmids. The pTK-CAT construct was the most sensitive to ICP4 activation, and the pAE-CAT construct was the least sensitive. The VP5 construct, although not activated by pEK4, was efficiently induced by cotransfection with pSG1, which encodes both ICP4 and ICP0 (data not shown).

Of the plasmids tested, only pgB-CAT was significantly induced by cotransfection with pBH27. CAT expression from pTK-CAT, pAE-CAT, and pVP5-CAT was essentially unaffected by cotransfection with pBH27, while CAT expression from pICP8-CAT appeared slightly inhibited (about two to threefold). These experiments indicate that, among the plasmids tested, transactivation by ICP27 is specific for the gB promoter-regulatory region.

ICP27 increased the amount of correctly initiated transcripts from pgB-CAT. The above experiments indicate that ICP27 increased the level of CAT enzyme expressed from pgB-CAT and its derivatives, but they did not address the mechanism by which ICP27 acts. If ICP27 stimulates transcription of the gB promoter or stabilizes mRNA transcribed from it, an increase in the amount of properly initiated gB-CAT transcripts should be seen. We therefore used an RNase protection assay to identify and quantitate mRNAs transcribed from pgB-CAT and its derivatives in transfected cells (Fig. 6A).

Cytoplasmic RNA was prepared from transfected cells, and equal amounts were annealed with a ³²P-labeled gB-

CAT antisense probe. As a marker, RNA prepared from cells transfected with pSV2-CAT (in which the simian virus 40 early promoter is fused to the CAT gene; 17) was also annealed to the antisense probe. In the latter case, only CAT-specific sequences, corresponding to a band of 152 nucleotides, were protected from digestion by RNase (Fig. 6B, lane 9). If transcription from pgB-CAT and its derivatives initiate at the expected site, then a protected band of approximately 193 nucleotides would be observed. An RNA species of this size was increased in response to ICP27. For the parental construct pgB-CAT, this band was absent in the absence of ICP27 (Fig. 6B, lane 3) but was induced to a low but detectable level by ICP27 (Fig. 6B, lane 4). The effect was more pronounced for the plasmid pgB-CAT-5' del(-175) (Fig. 6B, lanes 5 and 6). The plasmid pgB-CAT-s(-240), which showed a high level of basal CAT activity, also showed a high basal level of correctly initiated gB-CAT transcripts (Fig. 6B, lane 7). Furthermore, this level was increased by cotransfection of the ICP27 gene plasmid (Fig. 6B, lane 8), consistent with its induction in CAT activity. In cells transfected with pgB-CAT and its derivatives, a larger band of approximately 297 nucleotides, resulting from protection of the entire gB-CAT portion of the probe, was also observed. This band is presumably due to RNA transcripts which originate upstream of the gB cap site. In the experiment shown, this larger RNA species also appeared to be stimulated by ICP27, but in repeat experiments its induction was either less apparent or absent. Regardless of the response of the upstream transcript, our results demonstrate that ICP27 stimulates the accumulation of correctly initiated gB-CAT transcripts in transfected cells.

DISCUSSION

ICP27 specifically stimulates expression of the gB gene. The results we obtained in both infected and transfected cells indicate that the HSV-1 alpha protein ICP27 causes a stimulation of expression of at least one specific later viral gene, that encoding the glycoprotein gB. ICP27 is a phosphorylated nuclear protein which plays an essential but as yet undefined role in viral gene expression (49). The gB gene is also essential, encoding a virion envelope protein involved in cell penetration (52).

Expression of gB during HSV infection has previously been examined. In two studies it was found that gB mRNA levels rise significantly following viral DNA replication, suggesting that gB is regulated as a gamma 1 gene (22, 55). However, other studies have indicated that DNA replication has little, if any, effect on gB mRNA levels, consistent with beta gene regulation (21, 46). Our studies are also consistent with the regulation of gB as a beta gene, because the level of pgB protein synthesis did not appear to decrease in the presence of a DNA replication inhibitor (Fig. 1, compare lanes 7 and 9). In any case, our results suggest that regulation of the gB gene differs from that of several other HSV genes, both beta and gamma 1, because it shows a particular sensitivity to transactivation by ICP27.

Role of ICP27 during HSV-1 infection. Sacks et al. (49), using HSV ICP27 *ts* mutants, demonstrated that the normal accumulation of many viral early and late proteins is dependent on functional ICP27. We have observed that cells infected with these *ts* mutants at the nonpermissive temperature show an appreciable reduction in the accumulation of replicated viral DNA (S. Rice and D. Knipe, unpublished results). It was therefore unclear whether some of the

previously reported defects in *ts* mutant gene expression were due to an effect of ICP27 on gene expression or were secondary effects due to diminished DNA replication. To study more directly the effects of ICP27 on viral gene expression, we focused on expression at very early times after infection or on situations in which DNA replication was inhibited in both wild-type- and mutant-infected cells. Under these conditions, it was clear that *ts* defects in ICP27 resulted in a variable effect on the expression of beta and gamma 1 genes. For example, expression of the gB gene was reduced significantly, expression of the ICP8 gene was reduced more modestly, and expression of the ICP5 gene was reduced little if at all. At late times after infection, however, in the presence of ongoing viral DNA replication, it is clear that the full expression of most, if not all, gamma genes requires functional ICP27 (49).

We propose that ICP27 is involved in stimulating HSV-1 gene expression by two distinguishable mechanisms. First, ICP27 activates expression of HSV genes by a mechanism that is gene specific and independent of DNA replication. Second, ICP27 also activates gamma genes by a more general mechanism that is dependent on viral DNA replication. This second mode of gene activation may be due simply to the possibility that ICP27 stimulates viral DNA replication, which in turn would stimulate expression of gamma genes. Consistent with this idea, Challberg and colleagues (57) have recently shown that ICP27 stimulates DNA replication from a plasmid-borne HSV origin of replication in transfected cells. Superimposed on the second general mode of gene activation, ICP27 may also utilize the first mechanism of gene activation during the DNA replication phase of infection to specifically stimulate all or a subset of gamma genes independently of DNA replication.

The phenotype of the ICP27 *ts* mutants also suggests that ICP27 is involved in the negative regulation of alpha gene expression during HSV-1 infection. Sacks et al. (49), in long-term protein-labeling experiments, noted that cells infected with ICP27 *ts* mutants at the nonpermissive temperature accumulate higher than normal levels of ICP4 and ICP27 but not of ICP0. In our pulse-labeling experiments, we observed overexpression of ICP4, ICP27, and ICP0. Failure to detect overaccumulation of ICP0 in long-term labeling experiments may have been due to low stability of the ICP0 polypeptide.

We also observed a difference in the electrophoretic mobility of the ICP4 synthesized during a pulse-label at the nonpermissive temperature in cells infected with the ICP27 *ts* mutants. The ICP4 synthesized in the *ts* mutant-infected cells migrated on SDS-polyacrylamide gels as a more diffuse band with an overall decreased electrophoretic mobility. Although various studies have identified multiple electrophoretic forms of ICP4 in HSV-infected cells (37, 56), we have not yet attempted to relate the ICP4 species in our gels to previously identified forms of the protein. It is worth noting, however, that recent experiments from our laboratory have demonstrated that expression of ICP27 from a transfected plasmid results in a decrease in the electrophoretic mobility of ICP4 expressed from a cotransfected plasmid (L. Su and D. Knipe, unpublished data). Although it remains to be determined whether the apparent ability of ICP27 to affect ICP4 electrophoretic mobility is relevant to the control of HSV gene expression, it is noteworthy that a recent study has shown that the electrophoretic forms of ICP4 differ in their abilities to bind in complexes to specific HSV regulatory DNA sequences (35).

Effects of ICP27 in transfected cells. ICP27 expressed from

a transfected plasmid can induce expression of a chimeric gene in which the gB promoter-regulatory region has been fused to the CAT gene. Thus, HSV-1 encodes three alpha proteins, ICP4, ICP0, and ICP27, which on their own have the ability to enhance expression of a cotransfected gene. ICP27 differs from ICP4 and ICP0, which activate many HSV genes and promoters, in that stimulation by ICP27 is quite gene specific. This specificity suggests that ICP27 stimulates viral gene expression by a mechanism distinct from that of ICP4 or ICP0 and that a specific target nucleic acid sequence is required. Our results demonstrate that for the gB gene, these sequences lie between 175 bp upstream and 41 bp downstream of the gB transcriptional initiation site.

The mechanism by which ICP27 activates pgB-CAT expression in transfected cells is not known. However, induction of CAT activity correlates well with an increase in the level of correctly initiated gB-CAT transcripts. Therefore, ICP27 may stimulate the rate of transcription from the gB promoter or stabilize mRNAs transcribed from it. Preliminary mapping data indicates that HSV sequences 3' to the gB TATA element are not required for transactivation by ICP27 (S. Rice and D. Knipe, unpublished results). Thus, at present we favor a model in which ICP27 causes an increase in transcription, directly or indirectly.

Everett (10) recently reported that ICP27 could stimulate expression of a VP5-CAT construct in transfected cells but only in the presence of ICP4 and ICP0. Using similar but not identical plasmid constructs, we found that VP5-CAT expression can be stimulated by ICP4 and ICP0 but that the expression of ICP27 inhibits the transactivation induced by ICP4 and ICP0 (S. Rice, L. Su, and D. Knipe, unpublished data). A similar effect is seen with an ICP8-CAT construct (L. Su and D. Knipe, manuscript in preparation). Others (K. Leary, R. E. Sekulovich, and R. M. Sandri-Goldin, submitted for publication) have also recently noted the ability of ICP27 to repress gene expression in transfected cells. ICP27 thus appears similar to the adenovirus E1A proteins (36), which also can either positively or negatively affect gene expression in transient expression assays. For E1A, transactivation and transrepression appear to be mediated by different functional domains of the E1A polypeptides (29). Further work is required to better define the positive and negative regulatory effects of ICP27 and to determine if there are separable domains for these activities.

HSV encodes multiple trans-activators. HSV-1 encodes three alpha proteins which are capable of activating the expression of viral genes in transfected cells. In addition, the virus encodes a gamma 2 protein, alpha TIF, which is packaged in the virion and transactivates alpha genes early in infection (4, 41). The available data suggest that both ICP4 and alpha TIF stimulate transcription of their target genes, whereas the level at which ICP0 and ICP27 stimulate gene expression is not yet known. Genetic studies indicate that the genes encoding the viral transactivators, with the possible exception of the alpha TIF gene, are essential for efficient growth of the virus in cultured cells (8, 49, 50, 53). The interplay of these proteins thus may be critical to the correct expression of genes during the lytic infection.

Besides HSV-1, other herpesviruses also encode multiple transactivators. For example, Epstein-Barr virus encodes at least four proteins with transactivation potential (for a review, see reference 2). Interestingly, one of these proteins, BMLF1, shares a region of amino acid similarity with ICP27 (7). Human adenoviruses also appear to encode multiple transactivators (13, 19, 58). This may be a common feature

of large, nuclear-replicating DNA viruses which use the cellular machinery to carry out a regulated program of gene expression.

ACKNOWLEDGMENTS

We are grateful to Ed Wagner and Lishan Su for providing plasmids and to Roz Sandri-Goldin and Priscilla Schaffer for providing virus strains. Mike Greenberg provided expert help and advice with the RNase protection assay. We also acknowledge the excellent technical assistance of Melissa Bush. Finally, we thank Lishan Su, Min Gao, and Mike Greenberg for critical comments on the manuscript.

This research was supported by Public Health Service grant AI20530 from the National Institute of Allergy and Infectious Diseases. D.M.K. was supported by a Faculty Research Award from the American Cancer Society. S.A.R. was the recipient of Public Health Service research award F32 GM 11224 from the National Institutes of Health.

LITERATURE CITED

1. Ackermann, M., D. K. Braun, L. Pereira, and B. Roizman. 1984. Characterization of herpes simplex virus type 1 proteins 0, 4, and 27 with monoclonal antibodies. *J. Virol.* **52**:108-118.
2. Baichwal, V. R., and B. Sugden. 1988. Latency comes of age for herpesviruses. *Cell* **52**:787-789.
3. Bzik, D. J., B. A. Fox, N. A. Deluca, and S. Person. 1984. Nucleotide sequence specifying the glycoprotein gene, gB, of herpes simplex virus type 1. *Virology* **133**:301-314.
4. Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston. 1985. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for the stimulation of immediate-early transcription. *J. Mol. Biol.* **180**:1-19.
5. Clements, J. B., R. J. Watson, and N. M. Wilkie. 1977. Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. *Cell* **12**:275-285.
6. Costa, R. H., K. G. Draper, G. Devi-Rao, R. L. Thompson, and E. K. Wagner. 1985. Virus-induced modification of the host cell is required for expression of the bacterial chloramphenicol acetyltransferase gene controlled by a late herpes simplex virus promoter (VP5). *J. Virol.* **56**:19-30.
7. Davidson, A. J., and P. Taylor. 1987. Genetic relations between varicella-zoster virus and Epstein-Barr virus. *J. Gen. Virol.* **68**:1067-1079.
8. 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.
9. Everett, R. D. 1984. Trans-activation of transcription by herpesvirus products: requirement of two HSV-1 immediate-early polypeptides for maximum activity. *EMBO J.* **3**:3135-3141.
10. Everett, R. D. 1986. The product 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.* **68**:2507-2513.
11. Faber, S. W., and K. W. Wilcox. 1986. Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences in DNA. *Nucleic Acids Res.* **11**:1475-1489.
12. Gelman, I. H., and S. Silverstein. 1985. Identification of immediate-early genes from herpes simplex virus that transactivate the virus thymidine kinase gene. *Proc. Natl. Acad. Sci. USA* **82**:5265-5269.
13. Goding, C., P. Jalinot, D. Zajchowski, H. Boeuf, and C. Kedinger. 1985. Sequence-specific trans-activation of the adenovirus E1a early promoter by the viral E1V transcription unit. *EMBO J.* **4**:1523-1528.
14. Godowski, P. J., and D. M. Knipe. 1983. Mutations in the major DNA-binding protein of herpes simplex virus type 1 result in increased levels of viral gene expression. *J. Virol.* **47**:478-486.
15. Godowski, P. J., and D. M. Knipe. 1986. Transcriptional control of herpesvirus gene expression: gene functions required for

- positive and negative regulation. *Proc. Natl. Acad. Sci. USA* **83**:256–260.
16. Goldin, A. L., R. M. Sandri-Goldin, M. Levine, and J. C. Glorioso. 1981. Cloning of herpes simplex virus type 1 sequences representing the whole genome. *J. Virol.* **38**:50–58.
 17. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyl transferase in mammalian cells. *Mol. Cell. Biol.* **2**:1044–1051.
 18. Graham, F. L., and A. J. Van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**:456–467.
 19. Herrman, C. H., C. V. Dery, and M. B. Matthews. 1987. Transactivation of host and viral genes by the adenovirus E1B 19K tumor antigen. *Oncogene* **2**:25–36.
 20. Holland, L. E., K. P. Anderson, C. Shipman, and E. K. Wagner. 1980. Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNAs. *Virology* **101**:10–24.
 21. Holland, L. E., R. M. Sandri-Goldin, A. L. Goldin, J. C. Glorioso, and M. Levine. 1984. Transcriptional and genetic analyses of the herpes simplex virus type 1 genome: coordinates 0.29 to 0.45. *J. Virol.* **49**:947–959.
 22. Homa, F. L., T. M. Otal, J. C. Glorioso, and M. Levine. 1986. Transcriptional control signals of a herpes simplex virus type 1 late (γ_2) gene lie within bases –34 to +124 relative to the 5' terminus of the mRNA. *Mol. Cell. Biol.* **6**:3652–3666.
 23. Honess, R. W., and B. Roizman. 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* **14**:8–19.
 24. Honess, R. W., and B. Roizman. 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. *Proc. Natl. Acad. Sci. USA* **72**:1276–1280.
 25. Jones, P. C., and B. Roizman. 1979. Regulation of herpesvirus macromolecular synthesis. VIII. The transcription program consists of three phases during which both the extent of transcription and accumulation of RNA in the cytoplasm are regulated. *J. Virol.* **31**:299–314.
 26. Klessig, D. F., and C. W. Anderson. 1975. Block to multiplication of adenovirus serotype 2 in monkey cells. *J. Virol.* **16**:1650–1688.
 27. Knipe, D. M., D. Senechek, S. A. Rice, and J. L. Smith. 1987. Stages in the nuclear association of the herpes simplex virus transcriptional activator protein ICP4. *J. Virol.* **61**:276–284.
 28. Knipe, D. M., and A. E. Spang. 1982. Definition of a series of stages in the association of two herpesviral proteins with the cell nucleus. *J. Virol.* **43**:314–324.
 29. Lillie, J. W., M. Green, and M. R. Green. 1986. An adenovirus E1a protein region required for transformation and transcriptional repression. *Cell* **46**:1043–1051.
 30. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 31. Maniatis, T., S. Goodbourn, and J. A. Fischer. 1987. Regulation of inducible and tissue-specific gene expression. *Science* **236**:1237–1245.
 32. Mavromara-Nazos, P., M. Ackermann, and B. Roizman. 1986. Construction and properties of a viable herpes simplex virus recombinant lacking coding sequences of the $\alpha 47$ gene. *J. Virol.* **50**:807–812.
 33. Mavromara-Nazos, P., S. Silver, J. Hubenthal-Voss, J. C. McKnight, and B. Roizman. 1986. Regulation of herpes simplex virus type 1 genes: α gene sequence requirements for transient induction of indicator genes regulated by β or late (γ_2) promoters. *Virology* **149**:152–164.
 34. McKnight, S., and R. Tjian. 1986. Transcriptional selectivity of viral genes in mammalian cells. *Cell* **46**:795–805.
 35. Michael, N., D. Spector, P. Mavromara-Nazos, T. Kristie, and B. Roizman. 1988. The DNA-binding properties of the major regulatory protein $\alpha 4$ of herpes simplex virus. *Science* **239**:1531–1534.
 36. Moran, E., and M. B. Matthews. 1987. Multiple functional domains in the adenovirus E1A gene. *Cell* **48**:177–178.
 37. Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer. 1978. Anatomy of herpes simplex virus DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 $\times$ HSV-2 recombinants. *J. Virol.* **26**:389–410.
 38. O'Hare, P., and G. S. Hayward. 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular-weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. *J. Virol.* **53**:751–760.
 39. O'Hare, P., and G. S. Hayward. 1985. Three *trans*-acting regulatory proteins modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. *J. Virol.* **56**:723–733.
 40. Pereira, L., M. H. Wolff, M. Fenwick, and B. Roizman. 1977. Regulation of herpesvirus macromolecular synthesis. X. Properties of polypeptides made in HSV-1 and HSV-2 infected cells. *Virology* **77**:733–749.
 41. Post, L. E., S. Mackem, and B. Roizman. 1981. Regulation of genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. *Cell* **24**:555–565.
 42. Post, L. E., and B. Roizman. 1981. A generalized technique for deletion of specific genes in large genomes: α gene 22 of herpes simplex virus is not essential for growth. *Cell* **25**:227–232.
 43. Preston, C. M. 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant *tsK*. *J. Virol.* **29**:275–284.
 44. Preston, V. G., A. J. Davidson, H. S. Marsden, M. C. Timbury, J. H. Subak-Sharp, and N. M. Wilkie. 1978. Recombinants between herpes simplex virus types 1 and 2: analyses of genome structures and expression of immediate early polypeptides. *J. Virol.* **28**:499–517.
 45. Quinlan, M. P., and D. M. Knipe. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. *Mol. Cell. Biol.* **5**:957–963.
 46. Rafield, L. F., and D. M. Knipe. 1984. Characterization of the major mRNAs transcribed from the genes for glycoprotein B and DNA-binding protein ICP8 of herpes simplex virus type 1. *J. Virol.* **49**:960–969.
 47. Roizman, B., and W. Batterson. 1986. Herpesviruses and their replication, p. 607–636. *In* B. N. Fields and D. M. Knipe (ed.), *Fundamental virology*. Raven Press, New York.
 48. Roizman, B., and P. R. Roane, Jr. 1964. Multiplication of herpes simplex virus. II. The relation between protein synthesis and the duplication of viral DNA in infected HEP-2 cells. *Virology* **22**:262–269.
 49. Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer. 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. *J. Virol.* **55**:796–805.
 50. Sacks, W. R., and P. A. Schaffer. 1987. Deletion mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein ICP0 exhibit impaired growth in cell culture. *J. Virol.* **61**:829–839.
 51. Sandri-Goldin, R. M., M. Levine, and J. C. Glorioso. 1981. Method for induction of mutations in physically defined regions of the herpes simplex virus genome. *J. Virol.* **38**:41–49.
 52. Spear, P. J. 1985. Glycoproteins specified by herpes simplex virus, p. 351–356. *In* B. Roizman (ed.), *Herpesviruses*, vol. 3. Plenum Publishing Corp., New York.
 53. Stow, N. D., and E. C. Stow. 1986. Isolation and characterization of a herpes simplex virus type 1 mutant containing a deletion within the gene encoding the immediate early polypeptide Vmw 110. *J. Gen. Virol.* **67**:2571–2585.
 54. Watson, R. J., and J. B. Clements. 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. *Nature (London)* **285**:329–330.
 55. Weinheimer, S. P., and S. L. McKnight. 1987. Transcriptional and post-transcriptional controls establish the cascade of herpes simplex virus protein synthesis. *J. Mol. Biol.* **195**:819–833.
 56. Wilcox, K. W., A. Kohn, E. Sklyanskaya, and B. Roizman. 1980. Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides and can alter their affinity for DNA. *J. Virol.* **33**:167–182.

57. **Wu, C. A., N. J. Nelson, D. J. McGeoch, and M. D. Challberg.** 1988. Identification and mapping of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. *J. Virol.* **62**:435–443.
58. **Yoshida, K., L. Venkatech, M. Kuppuswamy, and G. Chinnadurai.** 1987. Adenovirus transforming 19kD T antigen has an enhancer-dependent trans-activation function and relieves enhancer repression mediated by viral and cellular genes. *Genes Devel.* **1**:645–658.
59. **Zinn, K., D. DiMaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human beta interferon gene. *Cell* **34**:865–879.