JOURNAL OF VIROLOGY, May 1997, p. 3580–3587
0022-538X/97/$04.00+0
Copyright © 1997, American Society for Microbiology
Vol. 71, No. 5

Differential Effects of Nerve Growth Factor and Dexamethasone on Herpes Simplex Virus Type 1 oriL- and oriS-Dependent DNA Replication in PC12 Cells

MARY ANN HARDWICKE† AND PRISCILLA A. SCHAFFER*

Division of Molecular Genetics, Dana-Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received 14 November 1996/Accepted 24 January 1997

The herpes simplex virus type 1 (HSV-1) genome contains three origins of DNA replication, one copy of oriL and two copies of oriS. Although oriL and oriS are structurally different, they have extensive nucleotide sequence similarity and can substitute for each other to initiate viral DNA replication. A fundamental question that remains to be answered is why the HSV-1 genome contains two types of origin. We have recently identified a novel glucocorticoid response element (GRE) within oriL that is not present in oriS and have shown by gel mobility shift assays that purified glucocorticoid receptor (GR), as well as GR present in cellular extracts, can bind to the GRE in oriL. To determine whether glucocorticoids and the GRE affect the efficiency of oriL-dependent DNA replication, we performed transient DNA replication assays in the presence and absence of dexamethasone (DEX). Because HSV-1 is a neurotropic virus and establishes latency in cells of neural origin, these tests were conducted in PC12 cells, which assume the properties of sympathetic neurons when differentiated with nerve growth factor (NGF). In NGF-differentiated PC12 cells, oriL-dependent DNA replication was enhanced 5-fold by DEX, whereas in undifferentiated cells, DEX enhanced replication ~2-fold. Notably, the enhancement of oriL function by DEX was abolished when the GRE was mutated. NGF-induced differentiation alone had no effect. In contrast to oriL, oriS-dependent DNA replication was reduced ~5-fold in NGF-differentiated PC12 cells and an additional 4-fold in differentiated cells treated with DEX. In undifferentiated PC12 cells, DEX had only a minor inhibitory effect (~2-fold) on oriS function. Although the *cis*-acting elements that mediate the NGF- and DEX-specific repression of oriS-dependent DNA replication are unknown, a functional GRE is critical for the DEX-induced enhancement of oriL function in NGF-differentiated PC12 cells. The enhancement of oriL-dependent DNA replication by DEX in differentiated PC12 cells suggests the possibility that glucocorticoids, agents long recognized to enhance reactivation of latent herpesvirus infections, act through the GRE in oriL to stimulate viral DNA replication and reactivation in terminally differentiated neurons in vivo.

The herpes simplex virus type 1 (HSV-1) genome contains three origins of DNA replication, one located in the unique long (U_L) region of the genome (oriL) and two within the repeats flanking the unique short (U_S) region of the genome (oriS) (26, 44, 46, 49, 52). The functional significance of the existence of three origins of replication in the pathogenesis of HSV-1 is not yet clear. Viruses with deletions in either oriL (37) or both copies of oriS (23) have no obvious growth defects in cultured cells, suggesting either that oriL or oriS is sufficient for viral replication in vitro.

oriL and oriS have extensive nucleotide sequence similarities, yet the two origins are structurally different. oriS consists of an imperfect 45-bp palindrome (45), whereas oriL is a perfect 144-bp palindrome (19, 38, 52). Both oriL and oriS are located in the promoter-regulatory regions of divergently transcribed genes. oriL is located within the promoter-regulatory regions of two early genes (UL29, which encodes the major single-stranded DNA binding protein, ICP8, and UL30, which encodes the DNA polymerase). oriS lies within the shared promoter-regulatory regions of the immediate-early genes encoding ICP4 and either ICP22 or ICP47.

* Corresponding author. Present address: Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104. Phone: (215) 573-9863. Fax: (215) 573-5344. E-mail: pschfr@mail.med.upenn.edu.

† Present address: SmithKline Beecham Pharmaceuticals, 1250 South Collegeville Rd., P.O. Box 5089, Collegeville, PA 19426.

Characterization of eukaryotic origins of DNA replication has revealed several common structural and functional features (10). Eukaryotic origins are composed of two discrete components: (i) a core which contains the binding site(s) of essential DNA initiation and elongation factors, the precise site of initiation, and elements which determine the direction of DNA synthesis and (ii) an auxiliary region(s) which contains the binding sites of transcription and other factors that enhance origin function but is dispensable under certain conditions. Characterization of the protein-DNA complexes that form with the core and auxiliary components of oriL and oriS has revealed similarities between these two origins and other eukaryotic origins and has begun to provide insight into the mechanism by which HSV-1 DNA replication is initiated (1, 7, 8, 53). Thus to date, three proteins or protein complexes have been shown to bind specifically to common sequences in the core components of oriL and oriS: a viral protein (origin binding protein [OBP]) that serves as an origin recognition factor (7, 9, 12–14, 29, 36, 50, 51), a second viral protein (OBPC) postulated to favor the switch from the theta to the rolling-circle mechanism of DNA replication and to facilitate encapsidation (2), and a cellular protein or protein complex, OF-1, whose binding to core elements is essential for efficient origin function (8). The promoter-regulatory regions surrounding oriL and oriS comprise the auxiliary regions of the two origins and contain binding sites for a variety of recognized transcription factors (22, 53). The results of systematic deletion mutagenesis suggest that these auxiliary regions and the proteins

that bind to them serve to enhance the efficiency of initiation in transient DNA replication assays when essential viral DNA replication factors are provided in *trans* (53). Despite our growing knowledge of the *cis*-acting elements and *trans*-acting factors involved in the initiation of DNA replication at oriL and oriS, studies of HSV-1 origins have revealed no significant functional differences between the two origins.

During studies designed to characterize oriL function, we identified a putative glucocorticoid response element (GRE) adjacent to the A-T-rich center of the oriL palindrome. Because of minor sequence variation in the region, oriS does not contain this element. In this report, we describe the formation of specific complexes between the oriL GRE and either purified glucocorticoid receptor (GR) or GR from cellular extracts. Transient DNA replication assays indicate that the GR-GRE interaction enhances oriL-dependent DNA replication in cells of neural lineage (PC12) terminally differentiated with nerve growth factor (NGF). Specifically, oriL-dependent DNA replication was ~5-fold greater in NGF-differentiated PC12 cells than in undifferentiated cells in the presence of a synthetic glucocorticoid, dexamethasone (DEX). In contrast, oriS-dependent DNA replication was reduced ~5-fold in NGF-differentiated PC12 cells and a further 4-fold in NGF-differentiated PC12 cells in the presence of DEX. DEX had only minor effects on oriL- and oriS-dependent DNA replication in undifferentiated PC12 cells.

The results of these studies demonstrate that oriL and oriS differ significantly in their responses to factors induced by NGF in cells of neural lineage and that DEX further enhances these differences. Although the mechanism responsible for the NGF- and DEX-induced repression of oriS function in PC12 cells is unclear, the DEX-induced enhancement of oriL function requires the GRE in oriL.

MATERIALS AND METHODS

Cells and virus. PC12 cells (a gift of John Wagner, Cornell University, Medical College, New York, N.Y.) were grown in Dulbecco's modified Eagle's medium (GibcoBRL Laboratories, Inc., Gaithersburg, Md.) supplemented with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.), 5% donor horse serum (JRH Biosciences, Lenexa, Kans.), 0.03% glutamine, 0.37% bicarbonate, 100 μg of streptomycin per ml, and 100 U of penicillin per ml at 37°C in 10% CO_2. To differentiate PC12 cells, NGF [NGF(2.5 S); Collaborative Biomedical Products, Bedford, Mass.] was added to cells at a concentration of 100 ng/ml for 6 days; NGF-containing medium was changed on day 3. The wild-type strain of HSV-1 (KOS) was grown and assayed as previously described (22).

Plasmids. Plasmids containing wild-type oriS (pOS-822) and oriL (pOL-833) have been described elsewhere (22, 53). Plasmid pOL GRE(−), containing a mutated oriL GRE, was generated as follows. Five contiguous oligonucleotides (containing the oriL palindrome and nucleotides on either side of the palindrome) and their complementary strands were synthesized with overlapping ends. Two point mutations in each hexanucleotide half-site of the GRE were engineered into two oligonucleotides, each containing one half-site of the complete GRE (see Fig. 2A). Each of the five oligonucleotides was annealed with its complementary strand by heating the paired oligonucleotides at 70°C for 3 min, followed by slow cooling to below 35°C. Two micrograms of each double-stranded oligonucleotide was then phosphorylated by incubation in 1 mM ATP, linker-kinase buffer (0.5 M Tris-Cl [pH 7.6], 0.1 M $MgCl_2$, 50 mM dithiothreitol [DTT], 1 mM spermidine HCl), and 1 U of T4 polynucleotide kinase (New England Biolabs, Beverly, Mass.) in a volume of 20 μl at 37°C for 1 h. The phosphorylated oligonucleotides were then ligated sequentially in linear order within the palindrome as follows: oligonucleotide 1 was ligated to oligonucleotide 2 in linker-kinase buffer with 40 U of T4 ligase (New England Biolabs) for 1 h at room temperature; oligonucleotides 4 and 5 were ligated in the same manner; ligated oligonucleotides 1 and 2 were then ligated to oligonucleotide 3; finally, ligated oligonucleotides 1, 2, and 3 were ligated to oligonucleotides 4 and 5 as described above except that the ligation was carried out at 16°C overnight. After the ligation was complete, the full-length 226-bp fragment was digested with *Bsr*GI and *Sgr*AI and isolated on a 5% polyacrylamide gel. The plasmid containing wild-type oriL, pOL833, was digested with *Bsr*GI and *Sgr*AI, and the 3.6-kb vector-containing fragment was isolated and gel purified. The 226-bp fragment containing the mutated GRE was ligated to the 3.6-kb vector-containing fragment to generate a plasmid containing 833 bp of oriL sequence with a mutated GRE. SURE cells (Stratagene, La Jolla, Calif.) were then transformed with the plasmid as previously described (22). The 226-bp region was sequenced as previously described to confirm the existence of the desired mutations and the absence of spurious mutations (22).

TABLE 1. Oligonucleotides used in this study

Half	Strand	Sequence
1st	1	5′-CATCCTGTTCGCACTTTGTCCTAATAAT-3′
	2	3′-GACAAGCGTGAAACAGGATTATTATATA-5′
2nd	1	5′-ATATATATTATTAGGACAAAGTGCGAACG-3′
	2	3′-TATAATAATCCTGTTTCACGCTTGCCTAG-5′

Transient DNA replication assays. For transient DNA replication assays, 100-mm-diameter dishes were seeded with 3.5×10^6 PC12 cells and incubated at 37°C in 10% CO_2. Eighteen to 24 h later, cells were transfected by the Lipofectin method. Specifically, plasmid DNA (10 μg) was diluted in 2 ml of serum-free medium. Lipofectin reagent (GibcoBRL) (final concentration, 8 μg/ml) diluted in 2 ml of serum-free medium was mixed with the diluted plasmid DNA; the mixture was held at room temperature for 15 min and then added dropwise to the culture medium. Monolayers were incubated at 37°C for 5 h, at which time they were rinsed once with serum-free medium and fresh medium containing serum was added. To differentiate transfected PC12 cells, NGF (100 ng/ml) was added to medium after the wash with serum-free medium. Untreated PC12 cells served as undifferentiated controls in all tests. After 3 days, fresh medium containing NGF (differentiated cells) or without NGF (undifferentiated cells) was added to the cells. On day 6 posttransfection, cells were infected with KOS at a multiplicity of 10 PFU/cell to provide essential DNA replication factors in *trans*. In experiments in which cells were treated with DEX, 0.5 μM DEX was added at the time of infection. Total cellular DNA was isolated 18 h after infection and purified as previously described (22). Ten micrograms of DNA was digested either with *Eco*RI and *Dpn*I or with *Eco*RI and *Mbo*I. Replicated DNA linearized with *Eco*RI was differentiated from unreplicated DNA by *Dpn*I digestion, whereas input DNA was separated from newly replicated DNA following *Mbo*I digestion. The DNAs were analyzed by Southern blot hybridization using ^{32}P-labeled, nick-translated vector sequence [pGEM7Zf(+)] as the probe as described previously (22). The resulting bands were quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, Calif.) and corrected to normalize for levels of DNA template.

Oligonucleotides and probes. The oligonucleotide probes used in the DNA binding assays (see Fig. 2) were synthesized by the Molecular Biology Core Facility, Dana-Farber Cancer Institute. To prevent the palindromic sequence from annealing to itself, the oriL GRE and the oriL/S GRE oligonucleotides were synthesized as four separate oligonucleotides. Each half of the full-length oligonucleotide was first annealed to its complementary strand (which was synthesized with a 5′ overhang) as previously described (22). The nucleotide sequences of the annealed oligonucleotides are shown in Table 1. The two annealed halves were then ligated, the double-stranded full-length molecules were gel purified, and radiolabeled probes were prepared as previously described (22). A double-stranded oligonucleotide containing the consensus binding site for nuclear factor 1 (NF-1) was used as a nonspecific competitor (7, 8). Nonspecific probes NSA, NSB, and site I oriS and oriL have been described elsewhere (7, 8). The GRE consensus oligonucleotide was purchased from Promega (Madison, Wis.).

Preparation of nuclear extracts and sources of GR protein. PC12 cells (10^7 to 10^8) were harvested in cold phosphate-buffered saline (PBS) and pelleted at $2,200 \times g$ and 4°C for 5 min. The cell pellet was resuspended in 5 ml of PBS and repelleted. Cells were resuspended in 1 ml of PBS, transferred to an Eppendorf tube, and repelleted at $3,000 \times g$ for 6 min at 4°C. The cell pellet was gently resuspended in 1 ml of reticulocyte standard buffer (RSB; 10 mM Tris-HCl [pH 7.5], 10 mM NaCl, 5 mM $MgCl_2$, 0.5 mM phenylmethylsulfonyl fluoride [PMSF], 0.5 mM DTT) and pelleted as before. Cells were again gently resuspended in RSB containing 0.5% Nonidet P-40 (NP-40), and the resulting nuclei were pelleted as before. Nuclei were resuspended in 1 ml of RSB without NP-40 and centrifuged as before. The final nuclear pellet was gently resuspended in 100 to 300 μl of buffer C (HEPES [pH 7.9], 25% glycerol, 0.42 M KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) and gently rocked at 4°C for 30 min, at which time the nuclear extract was transferred to an Oakridge tube and spun at $14,500 \times g$ for 30 min. The supernatant was then dialyzed against 300 ml of buffer D (20 mM HEPES [pH 7.9], 20% glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) for 1 h at 4°C with one change of buffer. Protein concentrations were determined for each sample by the method of Bradford (Bio-Rad, Hercules, Calif.), using a standard curve generated with bovine serum albumin as the protein source.

The plasmids expressing the GR DNA binding domain, GR440-525, and its mutated derivative, GR-EGA, were expressed in *Escherichia coli* by using the T7 system and purified to virtual homogeneity by Iris Alroy and Leonard Freedman (Sloan-Kettering Institute, Cornell University Graduate School of Medical Sciences, New York, N.Y.). The isolation and characterization of GR440-525 and GR-EGA have been described previously (1).

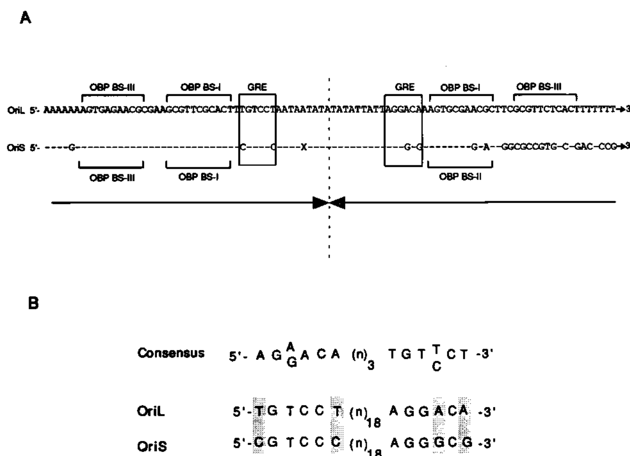


FIG. 1. Sequence comparison of oriL and oriS. (A) DNA sequence comparison of the OBP binding sites (OBP BS-I, -II, and -III) in oriL and oriS and the location of the GRE in oriL. The dashed lines in the oriS sequence indicate nucleotides that are identical in oriL. The "x" in the oriS sequence indicates a nucleotide that is not present in oriS. The boxes labeled GRE represent the bipartite hexanucleotide binding sites (half-sites) of the GRE present in oriL but not in oriS. The vertical dashed line and convergent arrows indicate the center of dyad symmetry of the two palindromic sequences. (B) Comparison of the sequence of the GRE present in oriL with the sequence of the consensus GRE. The consensus GRE consists of palindromic hexanucleotide half-sites with an intervening sequence consisting of three nucleotides of variable sequence. The GRE present in oriL also consists of two palindromic half-sites; however, the 5′ hexanucleotide in the consensus sequence is found 3′ in oriL. The sequence between the two half-sites in oriL is 18 nucleotides. The shaded boxes denote the nucleotides that are different in oriL and oriS.

Gel mobility shift assays. PC12 cell nuclear extracts (10 μg) were incubated at 25°C for 30 min with 4×10^4 to 8×10^4 cpm of probe (1 to 2 ng) and 1.5 μg of poly(dI-dC) · poly(dI-dC) (Pharmacia, Bern, Switzerland) in DNA binding buffer (10% glycerol, 50 mM HEPES [pH 7.5], 0.1 mM EDTA, 0.5 mM DTT, 100 mM NaCl, 1 mM TLCK) in a final volume of 10 μl. Protein-DNA complexes were resolved by electrophoresis on 6% nondenaturing acrylamide gels (37.5:1, acrylamide/bisacrylamide) at 4°C. In experiments involving antibody, the reaction was incubated for 5 min and the antibody was added for the remaining 25 min. Competition experiments were performed as described above; probe and unlabeled competitor DNAs were premixed prior to the addition of cell extracts in DNA binding buffer.

For gel mobility shift assays involving purified protein, GR440-525 and GR-EGA (as indicated in Fig. 2B) were incubated at 25°C for 30 min with 4×10^4 to 8×10^4 cpm of probe and 0.5 ng of poly(dI-dC) in binding buffer (10% glycerol, 20 mM Tris-HCl [pH 7.5], 1 mM EDTA, 0.05% NP-40, 50 mM KCl, 1 mM DTT) in a final volume of 10 μl. The resulting protein-DNA complexes were resolved as described above.

RESULTS

HSV-1 oriL contains a GRE. A computer search of the nucleotide sequence of the oriL palindrome for binding sites of transcription factors revealed the presence of a putative GRE hexanucleotide half-site immediately adjacent to the AT-rich center of the palindrome (18) (Fig. 1A). This element is identical to the half-site of a GRE identified in the promoter of the human metallothionein IIA gene (27, 28). Because oriL is a perfect palindrome, a second GRE hexanucleotide half-site is also present in the other arm of the palindrome, such that the oriL GRE consists of two half-sites separated by an 18-nucleotide spacer (Fig. 1A). Because nucleotide sequence differences occur in the analogous regions of the oriL and oriS palindromes, oriS does not contain a GRE (Fig. 1). The published consensus GRE consists of variations of the perfect palindrome 5′-AG(A/G)ACAN_3TGT(T/C)CT-3′ (15, 30) (Fig. 1B). The oriL GRE differs from the consensus GRE in two ways: (i) the two half-sites are switched such that the TGTCCT half-site is located 5′ to the AGGACA half-site, and (ii) the spacer between the two half-sites is 18 nucleotides rather than 3 (Fig. 1B).

Because the putative GRE in oriL differs from the consensus GRE, we were interested in determining whether the oriL GRE was able to bind GR. For this purpose, we performed gel shift analysis using two synthetic probes: a probe containing the wild-type oriL GRE (Fig. 2A) and a mutant probe in which the nucleotides at positions 1 and 6 of the first half-site were changed from T to C (as found in oriS) and nucleotides at positions 1 and 6 of the second half-site were changed from A to G (oriL/S GRE [Fig. 2A]). The probes were incubated with increasing concentrations of protein consisting of the purified GR DNA binding domain (GR440-525) or its mutated counterpart GR-EGA (1). The resulting protein-DNA complexes were separated on a 6% polyacrylamide gel.

Two complexes were observed with the wild-type probe (Fig. 2B, lanes 6 to 8), whereas no complex formation was observed with the mutant probe even at the highest concentration of GR tested (lanes 2 to 4). The specificity of complex formation was tested by incubating the wild-type (lanes 12 to 14) or mutant (lanes 9 to 11) probe with increasing concentrations of a purified mutant GR protein (GR-EGA), in which three amino acids had been changed to correspond to the analogous amino acids in the estrogen receptor (1). These tests demonstrated that (i) a region of GR containing the DNA binding domain can bind specifically to the wild-type oriL GRE but not to a mutated GRE and (ii) the wild-type GR binding domain, but not a mutated GR binding domain, can bind to the wild-type oriL GRE.

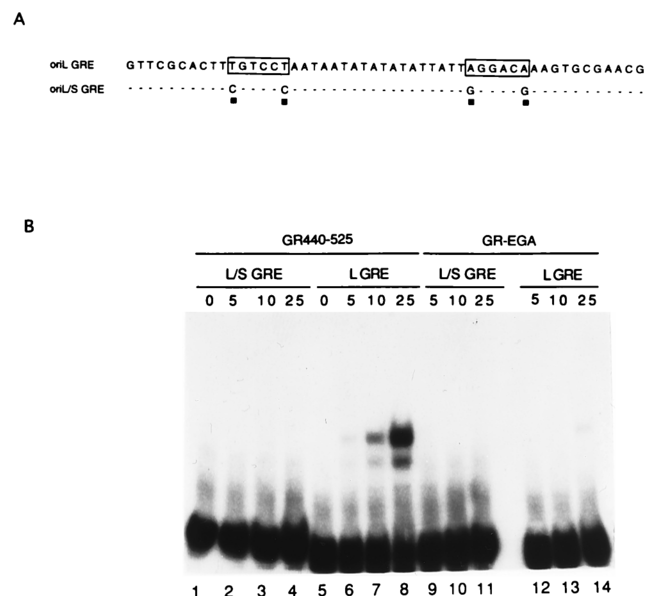


FIG. 2. Analysis of protein-DNA complex formation between the GRE in oriL and purified GR receptor. (A) Sequences of the oligonucleotide probes used in gel shift analysis. The boxes contain the two palindromic hexanucleotide half-sites of the oriL GRE. Shown below the oriL GRE is an oligonucleotide (oriL/S GRE) in which oriL nucleotides in positions 1 and 6 of the first hexanucleotide were changed from T to C and the nucleotides in positions 1 and 6 of the second hexanucleotide were changed from A to G. The dashes represent nucleotides that are identical in the two probes; the squares denote nucleotides that have been altered. (B) Radiolabeled oriL wild-type (L GRE; lanes 5 to 8) or mutant (L/S GRE; lanes 1 to 4) GRE-containing probes were incubated in the presence of increasing concentrations (0 to 25 mg) of purified GR DNA binding domain (GR440-525), and protein-DNA complexes were separated on a 6% polyacrylamide gel. The specificity of formation of this complex was confirmed by incubating radiolabeled L GRE (lanes 12 to 14) or L/S GRE (lanes 9 to 11) with increasing concentrations of purified mutant GR (GR-EGA) (lanes 9 to 14).

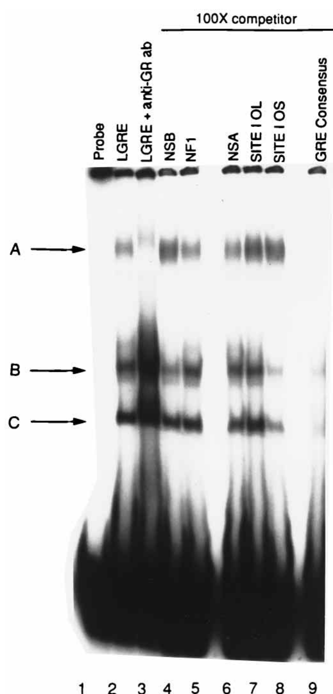


FIG. 3. Analysis of complex formation between oriL GRE and PC12 cell nuclear extracts. The radiolabeled oriL GRE-containing probe was incubated in the presence of PC12 cell nuclear extracts for 30 min, the resulting three complexes (labeled A, B, and C) were separated on a 6% polyacrylamide gel (lane 2). The specificity of formation of these complexes was demonstrated by incubating radiolabeled oriL GRE-containing probe and PC12 cell nuclear extracts with anti-GR antibody (ab) such that the complexes were supershifted (lane 3). To further confirm the specificity of complex formation, competition analysis was performed. PC12 cell nuclear extracts were incubated with radiolabeled oriL GRE-containing probe and a 100-fold excess of unlabeled competitor: lane 4 (NSB) and lane 6 (NSA), nonspecific sequences; lane 5, an oligonucleotide containing an NF-1 binding site; lanes 7 and 8, oligonucleotides containing OBP binding site I of oriL and oriS, respectively; lane 9, the GRE consensus oligonucleotide.

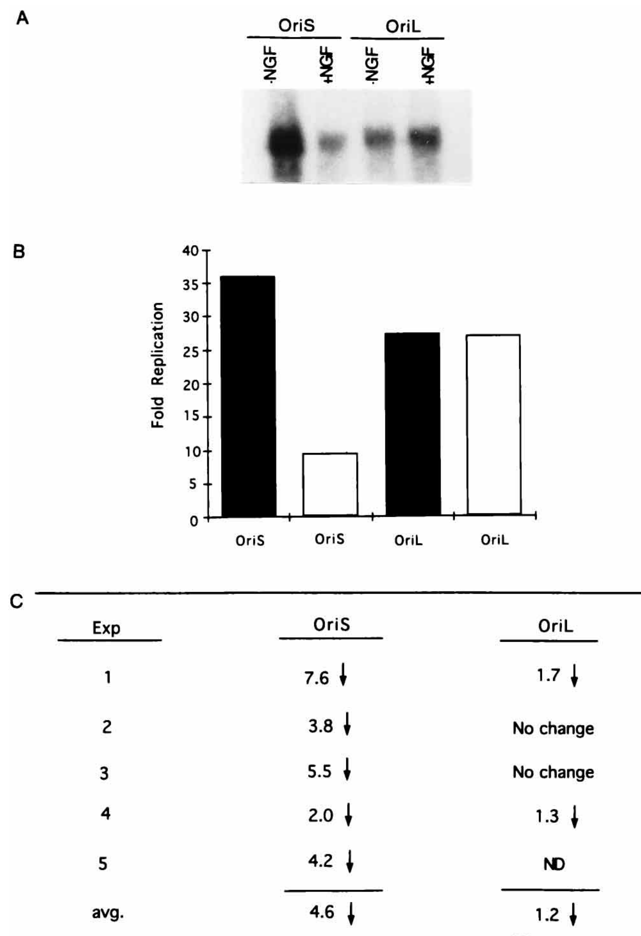
Functional analysis of oriL and oriS in PC12 cells. HSV-1 establishes latency in sympathetic neurons and reactivates with increased frequency in the presence of elevated levels of glucocorticoids (16, 21, 24, 32, 35, 43, 48). Based on these observations, we hypothesized that the GRE in oriL may function to enhance viral DNA replication in response to glucocorticoids specifically in cells of neural origin. We therefore tested the functional significance of the oriL GRE in PC12 cells, using oriS as a control. PC12 cells were derived from a transplantable rat pheochromocytoma and as such are of neural lineage (20). Important features of these cells are that following treatment with NGF, they develop neurites, become electrically excitable, and acquire a number of other properties characteristic of sympathetic neurons (20).

Before testing the effect of DEX on oriL- and oriS-dependent DNA replication in PC12 cells, we first determined whether the GRE in oriL can bind GR present in nuclear extracts of PC12 cells and whether treatment of PC12 cells with NGF affects the efficiency of oriL or oriS function in transient DNA replication assays.

GR in PC12 cells binds specifically to the GRE in oriL. The radiolabeled probe containing oriL GRE was incubated with nuclear extracts of PC12 cells, and the resulting protein-DNA complexes were visualized on a 6% polyacrylamide gel. Three major complexes were observed (Fig. 3, lane 2, arrows). The presence of GR in all three complexes was demonstrated by the ability of anti-GR-specific antibody to decrease the mobility of all three bands in the gel (lane 3). The slowest-migrating complex (A) was supershifted completely in the presence of anti-GR antibody, whereas only a portion of each of the two faster-migrating complexes (B and C) was supershifted. To test the binding specificity of complexes A, B, and C, competition analysis was performed. PC12 cell nuclear extracts were incubated with the probe containing oriL GRE and a 100-fold excess of unlabeled competitor probe (lanes 4 to 9). These tests demonstrated that only the probe containing the GRE consensus element was capable of competing completely for formation of complex A and competing partially for formation of complexes B and C (lane 9). Four nonspecific competitor probes, including two nonspecific oligonucleotide sequences, NSA and NSB (lanes 4 and 6), and probes containing an NF-1 binding site (lane 5) and the core binding sites for the HSV-1 OBP in oriL (lane 7), did not compete significantly for formation of the three complexes. The probe containing the core binding site for OBP in oriS competed to some degree for formation of complexes B and C but not for complex A; the reason for this is unclear. We conclude from these tests that GR in PC12 cell nuclear extracts is capable of forming specific complexes with the GRE in oriL.

NGF differentiation of PC12 cells inhibits oriS- but not oriL-dependent DNA replication. In a previous paper, we reported that oriL-dependent DNA replication was approximately 70% as efficient as oriS-dependent DNA replication in Vero cells. Similar results were obtained for undifferentiated PC12 cells in these studies (data not shown). To determine whether NGF differentiation of PC12 cells affected the efficiency of oriL- or oriS-dependent DNA replication, undifferentiated PC12 cells were transfected with either an oriL- or an oriS-containing plasmid. NGF was added to half of the cultures, and the transfected cells were allowed to differentiate for 6 days. Cells were then infected with HSV-1 strain KOS, and total cellular DNA was harvested after 18 h and assayed for plasmid replication. Replicated DNAs were quantitated by PhosphorImager scanning, and the resulting values were normalized for input DNA (data not shown). The replicated DNAs from one such experiment are shown in Fig. 4A. Results are presented in Fig. 4B as fold amplification of replicated plasmid DNA over input. The results of five independent experiments are presented in Fig. 4C as fold difference in the replication efficiencies of oriS- and oriL-containing plasmids in NGF-differentiated compared to undifferentiated PC12 cells. Although NGF treatment had no effect on the efficiency of oriL-dependent DNA replication in PC12 cells, a significant and reproducible decrease in the efficiency of oriS-dependent DNA replication (average decrease, 4.6-fold) occurred in response to NGF treatment.

DEX treatment of NGF-differentiated PC12 cells enhances oriL-dependent DNA replication and represses oriS-dependent DNA replication. Having shown that GR in PC12 cell extracts can bind to the GRE in oriL and that NGF differentiation of PC12 cells affects the efficiency of oriS- but not oriL-dependent DNA replication, we tested the effect of a synthetic glucocorticoid, DEX, on oriL- and oriS-dependent DNA replication in undifferentiated and NGF-differentiated PC12 cells. For this purpose, PC12 cells were transfected with either an oriL- or an oriS-containing plasmid, and half of each set of transfected cultures was treated for 6 days with NGF. All transfected cells were superinfected with HSV-1 on day 6, and DEX was added to half the cultures in each set at the time of superinfection. The results of four independent experiments in undifferentiated PC12 cells are shown in Table 2. DEX had only minor effects on origin function for oriS or oriL in undif-



Exp	OriS	OriL
1	7.6 ↓	1.7 ↓
2	3.8 ↓	No change
3	5.5 ↓	No change
4	2.0 ↓	1.3 ↓
5	4.2 ↓	ND
avg.	4.6 ↓	1.2 ↓

FIG. 4. Replication of oriL- and oriS-containing plasmids in undifferentiated (−NGF) and NGF-differentiated (+NGF) PC12 cells. (A) Southern blot analysis of a transient replication assay. PC12 cells were transfected with 10 μg of either an oriL-containing plasmid or an oriS-containing plasmid. Five hours after transfection, the cells were rinsed and medium with or without NGF (100 ng/ml) was added. Fresh medium was added on day 3. Six days after the addition of NGF, cells were infected with HSV-1 (multiplicity of infection 10 PFU/cell) in the appropriate medium (i.e., with or without NGF), and total cellular DNA was harvested 18 h postinfection. Ten micrograms of DNA was digested with either *Eco*RI and *Dpn*I to quantitate replicated DNAs (A) or *Eco*RI and *Mbo*I to quantitate input DNAs (data not shown). The digested DNAs were analyzed by Southern blot hybridization using a ^{32}P-labeled pUC19 probe. (B) Graph of data obtained from scanning the Southern blot shown in panel A. The replicated and input DNAs were quantitated by PhosphorImager scanning analysis and corrected for transfection efficiency. Results are presented as fold amplification over input DNA. Black bars represent replication assays in the absence of NGF; white bars represent the results of assays in the presence of NGF. (C) Results of five separate experiments performed as described for panel A. The replicated and input DNAs were quantitated by PhosphorImager scanning analysis, and the results are presented as fold difference in the replication efficiencies of either oriL- or oriS-containing plasmid in PC12 cells in the presence compared to the absence of NGF. The downward arrows represent the fold decrease in replication efficiency. avg., average change in the four or five experiments; ND, not determined.

ferentiated PC12 cells. Thus, the level of oriS-dependent DNA replication was slightly but reproducibly reduced, whereas replication from oriL was slightly but reproducibly increased in these tests.

In contrast to its effects in undifferentiated cells, the effects of DEX on plasmid replication in NGF-differentiated PC12 cells were marked (Table 3). Replication of the oriL-containing plasmid was ~5-fold greater in the presence compared to the absence of DEX, whereas replication of the oriS-containing plasmid was ~4-fold lower in the presence compared to the absence of DEX. This fourfold repression of oriS function by DEX was in addition to the 4- to 5-fold repression induced by NGF (Fig. 4).

TABLE 2. Fold differences in replication efficiencies of oriS- and oriL-containing plasmids in undifferentiated PC12 cells in the presence compared to the absence of DEX[a]

Expt	Fold difference	
	oriS	oriL
1	2.1 ↓	1.7 ↑
2	2.8 ↓	1.5 ↑
3	1.3 ↓	1.5 ↑
4	2.0 ↓	ND
Avg	2.1 ↓	1.6 ↑

[a] Undifferentiated PC12 cells were transfected with either an oriS or an oriL-containing plasmid as described in the legend to Fig. 4. At the time of superinfection, DEX was added to half of the cells, and at 18 h postinfection, total cellular DNA was harvested and processed as described in the legend to Fig. 4. The results of four separate experiments are presented as fold difference in replication efficiencies of either an oriL or an oriS-containing plasmid in undifferentiated PC12 cells in the presence compared to the absence of DEX. The arrows represent fold increase or decrease in replication efficiency. ND, not determined.

An oriL-containing plasmid mutated in the GRE does not respond to DEX in NGF-differentiated PC12 cells. The enhancing effect of DEX on the replication of the oriL-containing plasmid suggested strongly that the GRE in oriL serves as a target for GRs in response to DEX in NGF-differentiated PC12 cells. To test this hypothesis, we generated a mutant plasmid, pOL GRE(−), in which two point mutations were introduced into each half-site of the oriL GRE. These mutations were the same as those shown in the gel shift analysis to ablate the ability of purified GR to bind to the GRE (oriL/S GRE [Fig. 2A]). It should be noted that the point mutations generated in the first hexanucleotide half-site of the GRE in plasmid pOL GRE(−) are identical to the analogous region in oriS (compare Fig. 1A and 2A). The replication efficiencies of pOL and pOL GRE(−) were then tested in NGF-differentiated PC12 cells in the presence and absence of DEX. The average replication efficiencies of two independent experiments are shown in Fig. 5. As in previous tests (Table 3), DEX treatment produced a significant increase (~4.5-fold) in the replication efficiency of pOL which contains the wild-type

TABLE 3. Fold differences in replication efficiencies of oriS (pOS822)- and oriL (pOL833)-containing plasmids in NGF-differentiated PC12 cells in the presence compared to the absence of DEX[a]

Expt	Fold difference	
	oriS	oriL
1	5.7 ↓	2.6 ↑
2	2.6 ↓	4.4 ↑
3	3.9 ↓	4.3 ↑
4	ND	8.9 ↑
Avg	4.1 ↓	5.0 ↑

[a] Transient replication assays were performed in NGF-differentiated PC12 cells as described in the footnote to Table 2. The results of four separate experiments are presented as fold difference in replication efficiencies of either an oriL- or an oriS-containing plasmid in differentiated PC12 cells in the presence compared to the absence of DEX. The arrows represent fold increase or decrease in replication efficiency. ND, not determined.

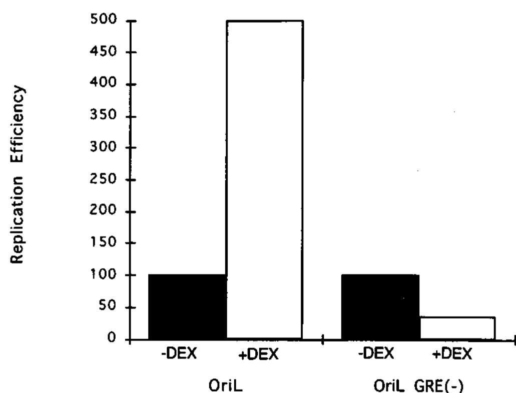


FIG. 5. Effect of DEX on a mutated oriL GRE plasmid in NGF-differentiated PC12 cells. PC12 cells were transfected with a plasmid containing either a wild-type oriL (pOL) or a plasmid containing four point mutations in oriL sequences, two in each hexanucleotide half-site of the GRE [pOL GRE(−)]. Replication efficiencies of the two plasmids were tested in NGF-differentiated PC12 cells in the presence and absence of DEX. The amplified DNA was quantitated by PhosphorImager scanning analysis, corrected for transfection efficiency. The replication efficiency of the wild-type oriL plasmid in NGF-differentiated PC12 cells in the absence of DEX was set to 100, and the replication efficiencies of the wild-type and mutant plasmids in NGF-differentiated PC12 cells in the presence or absence of DEX were calculated relative to this value. Black bars represent the replication efficiencies in differentiated PC12 cells in the absence of DEX; white bars represent the replication efficiencies in differentiated PC12 cells in the presence of DEX. These results represent the averages of two separate transfection experiments.

GRE. In contrast, no increase, but rather a reproducible decrease, in the replication efficiency of pOL (GRE−) occurred in the presence of DEX. The decrease in replication efficiency of plasmid pOL GRE(−) in response to DEX was similar to the decrease in replication efficiency of the plasmid containing the bona fide oriS observed in response to DEX in NGF-differentiated PC12 cells. The decrease in the replication efficiencies of both plasmids in response to DEX may reflect the fact that the GREs in the two plasmids differ by only two nucleotides.

Collectively, the results of these tests demonstrate that the increase in oriL-dependent DNA replication efficiency in the presence of DEX is due to the presence of the GRE in oriL and suggest that the activities of minor variants of the oriL GRE can be repressed by DEX in NGF-differentiated PC12 cells.

DISCUSSION

Differential effects of NGF and DEX on oriL- and oriS-dependent DNA replication. The functional significance of the existence of three origins of DNA replication in the HSV genome has been the subject of much speculation. oriL and oriS have considerable nucleotide sequence similarity and consequently have been postulated to function similarly. Indeed, in vitro tests of mutants lacking either oriL or both copies of oriS have shown that the two origins can substitute for one another (23, 37). The presence of a putative GRE in oriL but not oriS raised the possibility that this element mediates glucocorticoid-induced functional differences in the two origins. Because HSV is a neurotropic virus and establishes latency in sympathetic neurons, we chose to test the effects of DEX on oriL and oriS function in NGF-differentiated PC12 cells, which exhibit many of the properties of sympathetic neurons. Prior to testing the effects of DEX, however, it was necessary to test the effects of NGF-induced differentiation on oriL and oriS function. Unexpectedly, oriS-dependent DNA replication was significantly reduced in PC12 cells in response to NGF treatment and further reduced in NGF-differentiated cells in response to DEX. In contrast to oriS, NGF-induced differentiation of PC12 cells had no effect on oriL function, but DEX had a significant enhancing effect on oriL function in NGF-differentiated PC12 cells. Thus, the effects of DEX on both origins were observed in NGF-differentiated but not undifferentiated PC12 cells. DEX had no effect on the replication efficiency of an oriL-containing plasmid mutated in the GRE, demonstrating the specificity of the effect of DEX on oriL function.

Potential mechanisms of NGF-induced repression and DEX-induced enhancement of oriL and oriS function. A major experimental challenge will be to elucidate the mechanisms by which (i) NGF differentiation of PC12 cells represses oriS but not oriL function and (ii) DEX activates oriL and further represses oriS function in NGF-differentiated PC12 cells.

NGF is a member of the neurotrophin subfamily of growth factors, which is necessary for the survival and differentiation of sensory and sympathetic neurons (31). NGF mediates its growth- and differentiation-specific effects through a complex series of signal transduction pathways involving phosphorylation of preexisting proteins, some of which are transcription factors, and the induction of new sets of cellular proteins (reviewed in reference 47).

Although few studies have been conducted specifically in PC12 cells, glucocorticoids are known to exert their physiological effects on biological processes through GRs which act as ligand-dependent transcription factors. Glucocorticoid-GR complexes stimulate, or in some cases repress, gene expression by binding to GREs in the promoter-regulatory regions of genes regulated by glucocorticoids (11). GREs able to stimulate gene expression have been identified in the promoter-regulatory regions of Epstein-Barr virus, human immunodeficiency virus, mouse mammary tumor virus, Moloney murine sarcoma virus, and human papillomaviruses (3–5, 33, 34, 42).

At present, neither the molecular mechanism by which NGF differentiation of PC12 cells results in the repression of oriS function nor the time during differentiation when repression is first apparent is known. To answer these questions, the *cis*-acting elements and *trans*-acting factors that mediate repression must be identified. Efforts in this regard are currently under way.

The molecular basis for the inhibitory effect of DEX on oriS function in NGF-differentiated PC12 cells (Table 3) is also unknown but may involve the degenerate GRE in oriS. The basis for this suggestion is as follows: oriS contains a degenerate variant of the oriL GRE, and oriS function was inhibited by DEX in NGF-differentiated PC12 cells (Table 3); oriL GRE(−) differs from the degenerate GRE in oriS by only two nucleotides (Fig. 1 and 2), and the replication efficiency of oriL GRE(−) was also repressed by DEX in NGF-differentiated PC12 cells. Several published reports indicate that minor sequence variants of GREs can function as negative GREs (i.e., as transcriptional repressors) in response to glucocorticoids (11, 15, 30). Thus, it is possible that minor variations in the oriL GRE, such as found in oriS and in oriL GRE(−), correspond to negative GREs that respond negatively to glucocorticoids. However, the fact that the mutated GRE in oriL GRE(−) failed to bind purified GR in gel shift assays (L/S GRE [Fig. 2]) argues against the involvement of GR and these putative negative GREs in the DEX-induced repression of DNA replication driven by oriS and oriL GRE(−). It may be, however, that cellular transcriptional factors interact with GRs and generate the negative effects observed with the oriS and oriL GRE(−) plasmids. Evidence in support of this hypothesis comes from studies demonstrating that GRs can interfere with

transcriptional activators via protein-protein interactions and cause transcriptional repression (25, 40, 41, 54).

Is the DEX-induced enhancement of DNA replication from oriL linked to transcriptional activation? Although glucocorticoids are recognized transcriptional activators, this is the first report in which a GRE has been shown to be involved in the stimulation of origin-dependent DNA replication. Because the GRE in oriL and the degenerate GRE in oriS are also components of promoter-regulatory regions of genes transcribed divergently from these two origins, it may be that the enhancing and repressing effects of DEX on oriL and oriS function, respectively, are related to the transcriptional regulatory activities of DEX. In the case of oriL, it is possible that the DEX-induced enhancement of origin-dependent DNA replication is due, at least in part, to increased expression of the divergently transcribed genes encoding the single-stranded DNA binding protein, ICP8, and DNA polymerase, both of which are required for DNA replication. This possibility is currently being investigated.

Significance of NGF- and DEX-induced regulation of origin function for HSV-1 pathogenesis. A unique property of herpesviruses is their ability to cause both productive and latent infections of the host. During latency, viral genomes are maintained in the nuclei of sympathetic neurons for the lifetime of the host, greatly increasing the long-term survival capability of the virus. Reactivation and the resumption of productive infection following stress, trauma, and immunosuppression provide the opportunity for the virus to reactivate and spread to new hosts. Of special significance to the pathogenesis of HSV-1 are the facts that both NGF and DEX are induced in response to stress and that NGF is essential for the survival and functional integrity of sympathetic neurons in which HSV-1 establishes latent infections. Thus, the findings presented in this report indicate that HSV-1 utilizes factors needed for the survival of the host cell (in this case, NGF and DEX) to ensure its own survival and perpetuation.

We have shown recently that NGF treatment of PC12 cells activates expression from the promoter of the latency-associated transcripts which are thought to play a central role in the establishment and reactivation of latent HSV infections (16, 17). In this report, we have shown that NGF distinguishes between oriL and oriS, by repressing oriS but not oriL function. This observation suggests that factors present in NGF-differentiated but not undifferentiated neurons are responsible for this distinction and, further, that in NGF-differentiated neurons, oriL is the more active origin. One can speculate that the selective repression of oriS function in neurons confers a survival advantage on the virus, although no evidence to this effect is currently available.

Like NGF, DEX is induced in response to stress. Indeed, glucocorticoids are primarily responsible for suppression of the immune response following stress. It is well documented in the clinical literature and in animal models of HSV latency and reactivation that stress and the clinical administration of glucocorticoids result in immunosuppression and the reactivation of HSV infections (6, 21, 24, 32, 35, 43, 48). The fact that reactivation routinely follows immunosuppression has led to the concept that immunosuppression is the cause of reactivation (i.e., reactivation is controlled in immunocompetent individuals, whereas it is not controlled in the immunocompromised host). Although available evidence strongly supports a role for stress-induced, glucocorticoid-mediated immunosuppression in reactivation of HSV from latency, the targeted stimulation of oriL-dependent DNA replication by glucocorticoids may provide a critical boost in the reactivation process. From the perspective of the virus, the ability to reactivate in response to the very agents that induce immunosuppression confers a significant survival capability.

ACKNOWLEDGMENTS

We thank Iris Alroy and Leonard Freeman for the wild-type and mutant GR peptides, Paul Farrell for GR antibody, and Christine Dabrowski, Rob Jordan, Eleanor Mendoza, and Anh Nguyen-Huynh for helpful discussions and thoughtful review of the manuscript.

This work was supported by Public Health Service grant ROIA128537 from the National Institute of Allergy and Infectious Diseases. M.A.H. was the recipient of postdoctoral fellowship F32AI08878 from the National Institutes of Health.

REFERENCES

1. **Alroy, I., and L. P. Friedman.** 1992. DNA binding analysis of glucocorticoid receptor specificity mutants. Nucleic Acids Res. **20:**1045–1052.
2. **Baradaran, K., M. A. Hardwicke, C. E. Dabrowski, and P. A. Schaffer.** 1996. Properties of the novel herpes simplex virus type 1 origin binding protein, OBPC. J. Virol. **70:**5673–5679.
3. **Bauer, G.** 1983. Induction of Epstein-Barr virus early antigens by corticosteroids: inhibition by IPA and retinoic acid. Int. J. Cancer **31:**291–295.
4. **Buetti, E., and H. Diggelmann.** 1981. Cloned mouse mammary tumor virus DNA is biologically active in transfected mouse cells and its expression is stimulated by glucocorticoid hormones. Cell **23:**335–345.
5. **Chan, W. K., G. Klock, and H. U. Bernard.** 1989. Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. J. Virol. **3:**3261–3269.
6. **Cook, S. D., M. J. Paveloff, J. J. Doucet, A. J. Cottingham, F. Sederati, and J. M. Hill.** 1991. Ocular herpes simplex virus reactivation in mice latently infected with latency-associated transcript mutants. Invest. Ophthalmol. Visual Sci. **32:**1558–1561.
7. **Dabrowski, C. E., and P. A. Schaffer.** 1991. Herpes simplex virus type 1 origin-specific binding protein: oriS-binding properties and effects on cellular proteins. J. Virol. **65:**3140–3150.
8. **Dabrowski, C. E., P. C. Carmillo, and P. A. Schaffer.** 1994. Cellular protein interactions with herpes simplex virus type 1 oriS. Mol. Cell. Biol. **14:**2545–2555.
9. **Deb, S., and S. P. Deb.** 1989. Analysis of oriS sequence of HSV-1: identification of one functional DNA binding domain. Nucleic Acids Res. **17:**2733–2752.
10. **DePamphilis, M. L.** 1988. Transcriptional elements as components of eukaryotic origins of DNA replication. Cell **52:**635–638.
11. **Diamond, M. I., J. N. Miner, S. K. Yoshinaya, and K. R. Yamamoto.** 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science **249:**1266–1272.
12. **Elias, P., and I. R. Lehman.** 1988. Interaction of origin binding protein with an origin of replication of herpes simplex virus 1. Proc. Natl. Acad. Sci. USA **85:**2959–2963.
13. **Elias, P., C. M. Gustafsson, and O. Hammarsten.** 1990. The origin binding protein of herpes simplex virus 1 binds cooperatively to the viral origin of replication ori. J. Biol. Chem. **265:**17167–17173.
14. **Elias, P., M. E. O'Donnell, E. S. Mocarski, and I. R. Lehman.** 1986. A DNA binding protein specific for an origin of replication of herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA **83:**6322–6326.
15. **Evans, R. M.** 1988. The steroid and thyroid hormone receptor superfamily. Science **240:**889–895.
16. **Frazier, D. P., D. Cox, E. M. Godshalk, and P. A. Schaffer.** 1996. Identification of *cis*-acting sequences in the promoter of the herpes simplex virus type 1 latency-associated transcripts required for activation by nerve growth factor and sodium butyrate in PC12 cells. J. Virol. **70:**7433–7444.
17. **Frazier, D. P., D. Cox, E. M. Godshalk, and P. A. Schaffer.** 1996. The herpes simplex virus type 1 latency-associated transcript promoter is activated through Ras and Raf by nerve growth factor and sodium butyrate in PC12 cells. J. Virol. **70:**7424–7432.
18. **Gloss, B., H. U. Bernard, K. Seedorf, and G. Klock.** 1987. The upstream regulatory region of human papillomavirus 16 contains an E2 protein independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. EMBO J. **6:**3735–3743.
19. **Gray, C. P., and H. C. Kaerner.** 1984. Sequence of the putative origin of DNA replication in the U_L region of herpes simplex virus type 1 ANG DNA. J. Gen. Virol. **65:**2109–2119.
20. **Greene, L. A., and A. S. Tischler.** 1976. Establishment of a nonadrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA **73:**2424–2428.
21. **Halford, W. P., B. M. Gebhardt, and D. J. J. Carr.** 1996. Mechanisms of herpes simplex virus type 1 reactivation. J. Virol. **70:**5051–5060.
22. **Hardwicke, M. A., and P. A. Schaffer.** 1995. Cloning and characterization of herpes simplex virus type 1 oriL: comparison of replication and protein-

DNA complex formation by oriL and oriS. J. Virol. **69:**1377–1388.
23. **Igarashi, K., R. Fawl, R. J. Roller, and B. Roizman.** 1993. Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. J. Virol. **67:**2123–2132.
24. **Inglis, A. F.** 1993. Herpes simplex virus infection: a rare case of prolonged croup. Arch. Otolaryngol. Head Neck Surg. **119:**551–552.
25. **Jonat, C., H. J. Rahmsdorf, K. K. Park, A. C. Cato, S. Gebel, H. Ponta, and P. Herrlich.** 1990. Antitutmore promotion and antiinflammation: downmodulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell **62:** 1189–1204.
26. **Kaerner, H. C., I. B. Maichle, A. Ott, and C. H. Schrôder.** 1979. Origins of two different classes of defective HSV-1 Angelotti DNA. Nucleic Acids Res. **6:**1467–1478.
27. **Karin, M., and R. Richards.** 1982. Human metallothionein genes—primary structure of the metallothionein-II gene and a related processed gene. Nature **299:**797–802.
28. **Karin, M. A., ●. Haslinger, H. Holtgreve, R. I. Richards, P. Krauter, H. M. Westphal, and M. Beato.** 1984. Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIA gene. Nature **308:**513–9.
29. **Koff, A., and P. Tegtmeyer.** 1988. Characterization of major recognition sequences for a herpes simplex virus type 1 origin-binding protein. J. Virol. **62:**4096–4103.
30. **Kupfer, S. R., and W. C. Summers.** 1990. Identification of a glucocorticoid-responsive element in Epstein-Barr virus. J. Virol. **64:**1984–1990.
31. **Levi-Montcalcini, R.** 1987. The nerve growth factor: thirty-five years later. EMBO J. **6:**1145–1154.
32. **McCinn, P. C., I. S. Lim, P. E. McKenzie, A. G. van Deth, and A. Simmons.** 1989. Disseminated herpes simplex virus infection in an apparently immunocompetent woman. Med. J. Aust. **151:**588–594.
33. **Miksicek, R., A. Heber, W. Schmid, U. Danesch, G. Posseckert, M. Beato, and G. Schutz.** 1986. Glucocorticoid responsiveness of the transcriptional enhancer of Moloney murine sarcoma virus. Cell **46:**283–290.
34. **Moens, U., N. Subramaniam, B. Johansen, T. Johansen, and T. Traavik.** 1994. A steroid hormone response unit in the late leader of the noncoding control region of the human polyomavirus BK confers enhanced host cell permissivity. J. Virol. **68:**2398–2408.
35. **Mosimann, F., P. F. Cuenoud, F. Steinhauslen, and G. P. Wauters.** 1994. Herpes simplex esophagitis after renal transplantation. Transplant. Int. **7:**79–82.
36. **Olivo, P. D., N. J. Nelson, and M. D. Challberg.** 1988. Herpes simplex virus DNA replication: the UL9 gene encodes an origin-binding protein. Proc. Natl. Acad. Sci. USA **85:**5414–5418.
37. **Polvino-Bodnar, M., P. K. Orberg, and P. A. Schaffer.** 1987. Herpes simplex virus type 1 oriL is not required for virus replication or for the establishment and reactivation of latent infection in mice. J. Virol. **61:**3528–3535.
38. **Quinn, J. P., and D. J. McGeoch.** 1985. DNA sequence of the region in the genome of herpes simplex virus type 1 containing the genes for DNA polymerase and DNA binding protein. Nucleic Acids Res. **13:**8143–8163.
39. **Rock, D., J. Lokensgard, T. Lewis, and G. Kutish.** 1992. Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. J. Virol. **66:**2484–2490.
40. **Sakai, D. D., S. Helms, J. Carlstadt-Duke, J. A. Gustafsson, F. M. Rottman, and K. R. Yamamoto.** 1988. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev. **2:**1144–1154.
41. **Schåle, R., P. Rangarajan, S. Kliewer, L. J. Ransone, J. Bolado, N. Yang, I. V. Verma, and R. M. Evans.** 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. Cell **62:**1217–1226.
42. **Schuster, C., S. Chasserot-Golaz, and G. Beck.** 1991. Activation of Epstein-Barr virus promoters by a growth-factor and a glucocorticoid. FEBS Lett. **284:**82–86.
43. **Shane, S. A., M. W. Wollman, and D. Claassen.** 1994. Herpes simplex dissemination following glucocorticoids for upper airway obstruction in an adolescent girl. Pediatr. Emerg. Care **10:**160–162.
44. **Spaete, R. R., and N. Frenkel.** 1985. The herpes simplex virus amplicon: analyses of cis-acting replication functions. Proc. Natl. Acad. Sci. USA **82:** 694–698.
45. **Stow, N., and E. C. McMonagle.** 1983. Characterization of the TR_SIR_S origin of DNA replication of herpes simplex virus type 1. Virology **130:**427–438.
46. **Stow, N. D.** 1982. Localization of an origin of DNA replication within the TRs/IRs repeated region of the herpes simplex virus type 1 genome. EMBO J. **1:**863–867.
47. **Szeberenyi, J., and P. Eberhardt.** 1994. Cellular components of nerve growth factor signaling. Biophys. Biochim. Acta **1222:**187–202.
48. **Underwood, G. E., and S. D. Weed.** 1974. Recurrent cutaneous herpes simplex in hairless mice. Infect. Immun. **10:**471–474.
49. **Vlazny, D. A., and N. Frenkel.** 1981. Replication of herpes simplex virus DNA: localization of replication signals within defective virus genomes. Proc. Natl. Acad. Sci. USA **78:**742–746.
50. **Weir, H. M., and N. D. Stow.** 1990. Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the oriS replication origin. J. Gen. Virol. **71:**1379–1385.
51. **Weir, H. M., J. M. Calder, and N. D. Stow.** 1989. Binding of the herpes simplex virus type 1 UL9 gene product to an origin of viral DNA replication. Nucleic Acids Res. **17:**1409–1425.
52. **Weller, S. K., A. Spadaro, J. E. Schaffer, A. W. Murray, A. M. Maxam, and P. A. Schaffer.** 1985. Cloning, sequencing, and functional analysis of oriL, a herpes simplex virus type 1 origin of DNA synthesis. Mol. Cell. Biol. **5:**930–942.
53. **Wong, S. W., and P. A. Schaffer.** 1991. Elements in the transcriptional regulatory region flanking herpes simplex virus type 1 oriS stimulate origin function. J. Virol. **65:**2601–2611.
54. **Yang-Yen, H. F., J. C. Chambard, Y. L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin.** 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell **62:**1205–1215.