
Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences in DNA

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ABSTRACT

We report that the herpes simplex virus (HSV) transcription regulatory protein designated ICP4 is a component of a stable complex between protein and specific nucleotide sequences in double-stranded DNA formed by addition of exogenous DNA to either a crude extract obtained from HSV-1 infected cells or a partially purified preparation of native ICP4. DNA sites which are bound directly or indirectly to ICP4 have been designated ICP4/protein binding sites. Three independent ICP4/protein binding sites have been identified by DNase footprinting; two are in the vector pBR322 and one is located approximately 100 nucleotides upstream from the HSV glycoprotein D mRNA cap site. Comparison of the nucleotide sequences in these three sites reveals several regions of homology. We propose that the sequence 5'-ATCGTCNNNNYCGRC-3' (N = any base; Y = pyrimidine; R = purine) forms an essential component of the ICP4/protein binding site.

INTRODUCTION

The genome of herpes simplex virus (HSV) is a linear, double-stranded DNA molecule containing approximately 150 kilobase pairs and at least 70 genes. Current data suggest that each gene has a separate promoter (1) and is transcribed as a separate unit by the host RNA polymerase II (2). The temporal order of viral gene transcription is regulated by both trans-acting viral factors (3, 4) and cis-acting nucleotide signals (5-9). These regulatory elements influence the extent to which a viral gene is transcribed during each developmental phase of productive infection (10).

At least three different HSV-encoded transcriptional regulatory proteins have been identified (3, 11-15). One of these viral factors is a polypeptide designated ICP4 (16), VP175 (17), or Vmw175 (18). ICP4 is a phosphorylated polypeptide with an apparent molecular weight of 165,000 to 175,000 as estimated by gel electrophoretic mobility (19, 20) or 132,835 based on DNA

sequence analysis (21). It is synthesized at a maximum rate during the immediate early phase (4 to 6 hr) of viral infection and is rapidly translocated to the nucleus (22). When extracted with high salt from infected cell nuclei, the native protein is a homodimer (23). Relatively impure preparations of ICP4 bind to single-stranded DNA (24) and double-stranded DNA (24a).

The role of ICP4 in the regulation of viral gene expression has been established by analyses of the phenotypic properties of viral mutants with lesions in the gene encoding ICP4 (25-27) and by transient expression assays (11-15, 28-31). Results from these studies indicate that ICP4 functions as a positive regulatory factor to promote transcription from certain early and delayed early viral genes. ICP4 may also act as a negative regulatory factor to inhibit transcription from certain immediate early genes (13, 27). There are no firm conclusions regarding which components of the transcriptional apparatus are affected by ICP4. It has been suggested (5) that ICP4 modulates viral transcription by (i) binding to specific viral DNA sequences, (ii) modifying cellular transcription factors, or (iii) inactivating repressors.

We have been interested in elucidating the mechanism whereby ICP4 regulates transcription of specific viral genes. In a previous report (23), we described a method for partially purifying native ICP4. In preliminary experiments with this preparation and a modification of the DNA binding immunoassay described by McKay (32), we detected an association between ICP4 and recombinant DNAs consisting of HSV DNA fragments linked to a procaryotic vector. In control experiments, we were surprised to observe that ICP4 was a component of a stable complex between protein and specific DNA fragments derived from the vector pBR322. The availability of a fully-sequenced plasmid molecule which contained specific sites that associated either directly or indirectly with ICP4 prompted us to investigate this finding in more detail.

In this paper we report that a stable protein-DNA complex which includes ICP4 and a specific sequence of nucleotides within the tet gene of the procaryotic vector pBR322 can be formed in vitro. We also report that a sequence of nucleotides upstream from the HSV-1 glycoprotein D (gD) gene mRNA cap site can form a

stable association with ICP4. Although our results demonstrate that ICP4 is a component of a complex between protein and DNA, the data do not reveal whether the association between ICP4 and DNA is mediated by ICP4 itself or by another protein. Therefore we shall refer to DNA sites which are associated with ICP4 as "ICP4/protein binding sites". The ICP4/protein binding site in the gD gene shares extensive homology with the ICP4/protein binding sites in the tet gene of pBR322.

MATERIALS AND METHODS

Cells and viruses. Uninfected and infected Vero cells were cultured as described previously (23). The virus strains used were HSV-1(HFEM) and HSV-2(G).

Crude cell extracts. Vero cells were infected with HSV-1(HFEM) or HSV-2(G) at an moi of 10 at 37°C for either 6 or 16 hrs and then harvested. Cells were scraped into ice cold phosphate buffered saline supplemented with 0.6 mM EDTA and 0.1 mM L-1-tosylamide-2-phenylmethyl chloromethyl ketone (TPCK), pelleted, and then suspended at 2×10^7 cells/ml in ice cold lysis buffer (50 mM Tris-HCl pH 8.0, 500 mM NaCl, 2% Nonidet P40 (NP40)). After 30 min on ice, the lysate was clarified by centrifugation at 12,000 x g for 10 min. The supernatant was stored at -80°C until use.

Partial purification of ICP4. A preparation of fraction VII (ref. 23) from 1×10^{10} Vero cells infected with HSV-1(HFEM) was dialyzed against TEG buffer (10 mM Tris-HCl pH 8.0, 1.0 mM EDTA, 10 mM β -mercaptoethanol, 10% glycerol) supplemented with 25 mM $(\text{NH}_4)_2\text{SO}_4$ and loaded onto a DEAE-cellulose column (1.4 x 18 cm) equilibrated in TEG buffer plus 25 mM $(\text{NH}_4)_2\text{SO}_4$. The column was washed with 50 ml of this equilibration buffer. Proteins were eluted with a 25 to 300 mM linear gradient of $(\text{NH}_4)_2\text{SO}_4$ in TEG buffer(100 ml). The ICP4 peak was located by radioimmunoassay (8), pooled, and precipitated by addition of $(\text{NH}_4)_2\text{SO}_4$ to 50% of saturation. The pellet was resuspended and stored as fraction VIII at -20°C in 10 mM Tris-HCl pH 8.0, 1.0 mM EDTA, 10 mM β -mercaptoethanol, 50% (v/v) glycerol, and 100 mM $(\text{NH}_4)_2\text{SO}_4$.

Preparation of DNA. Uniformly radiolabeled plasmid DNA was extracted (33) from Escherichia coli after overnight culture in low phosphate labeling medium (70 mM Tris-HCl pH 7.5, 8 mM NaCl,

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20 mM NH₄Cl, 2.5 mM MgSO₄, 0.1 mM CaCl₂, 1.0 mM KCl, 0.1 mM KH₂PO₄, 5.5 mM glucose, 1 µg/ml thiamine, 0.2%(w/v) casein acid hydrolysate (Colab) supplemented with 35 µCi/ml [³²P]ortho-phosphate (carrier free, New England Nuclear) and the appropriate antibiotic. The initial specific activity of the DNA was approximately 1 x 10⁶ dpm/µg. Salmon sperm DNA (Worthington) was further purified by phenol extraction and ethanol precipitation. DNAs were cleaved with restriction endonucleases according to the supplier's instructions.

DNA binding immunoassay. All operations were carried out at 22°C unless indicated otherwise. Radiolabeled target DNA and nonlabeled carrier DNA were added as indicated to DNA binding buffer (DBB) (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 0.5mM EDTA, 2 mM β-mercaptoethanol, 50 µg/ml nuclease free BSA). A sample of a crude cell extract or fraction VIII was added and the mixture (typically 50 µl) was incubated for 30 min. Antibody (2 µl) was then added and the mixture was incubated for an additional 15 to 30 min. A 10% suspension (50 µl) of killed Staphylococcus aureus (Pansorbin, Calbiochem-Behring) in immunoprecipitation buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP40, 0.05% sodium deoxycholate, 1.5 mg/ml BSA) was added and the mixture was incubated for an additional 15 min. The immunoprecipitate was pelleted at 12,000 x g for 1 min, washed once in 100 µl of wash buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.3% NP40, 0.3% sodium deoxycholate) and resuspended in 15 µl of DNA elution buffer (90 mM Tris-borate pH 8.3, 10 mM EDTA, 2% SDS, 0.3M β-mercaptoethanol, 10% glycerol, 0.25 mg/ml bromophenol blue). The sample was heated for 5 min at 65°C and then centrifuged at 12,000 x g for 3 min to remove the S. aureus.

Gel electrophoresis. Double-stranded DNA fragments were suspended in DS buffer (90 mM Tris-borate pH 8.3, 50 mM EDTA, 3% Ficoll (type 400), 0.25 mg/ml bromophenol blue) and resolved by electrophoresis at constant voltage through polyacrylamide gels (5% acrylamide, 0.25% bisacrylamide) prepared and run in 90 mM Tris-borate pH 8.3, 2.5 mM EDTA, 0.2% SDS. Single-stranded DNA fragments were suspended in SS buffer (80% (v/v) formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue) and resolved by electrophoresis at constant power through 8%

acrylamide-8.3M urea sequencing gels (34). Radiolabeled DNA was detected by exposure of the dried gels to X-ray film.

Radiolabeled DNA for footprints. Purified plasmid DNA was cleaved with a restriction endonuclease, treated with calf intestine alkaline phosphatase (Boehringer-Mannheim), and then labeled at the 5' end with *E. coli* polynucleotide kinase (New England Biolabs) and [³²P]ATP (New England Nuclear). The DNA was then digested with a second restriction endonuclease and electrophoresed through a preparative polyacrylamide gel (6.5% acrylamide, 0.32% bisacrylamide). The appropriate end-labeled fragment was purified from a gel slice by electroelution in low conductivity buffer (5 mM Tris, 2.5 mM acetic acid, 1 mM EDTA, 0.1% SDS) as described by McDonnell et al (35). Except where indicated, these procedures were carried out as described by Maxam and Gilbert (34).

DNase footprint experiments. Double-stranded DNA fragments (10 to 20 ng) labeled at one 5' end with [³²P]orthophosphate were incubated in modified DBB (lacking MgCl₂) for 30 min at 22°C with a sample of fraction VIII (0 to 6.4 µl). The reaction mixture (100 µl) was then supplemented to contain 5 mM MgCl₂, 1 mM CaCl₂, and DNase I (12.5 to 100 ng) and incubated for 2 min at 22°C. An equal volume of stop buffer (2.0M ammonium acetate, 75 mM EDTA, 50 µg/ml tRNA) was then added. The nucleic acids were sequentially extracted with phenol and chloroform and then precipitated with ethanol. The dried pellets were suspended in SS buffer (15 to 20 µl) and analyzed on acrylamide-urea gels.

RESULTS

Detection of Specific Protein-DNA complexes containing ICP4.

Previous reports have established that stable protein-DNA complexes containing ICP4 can be formed in vitro (24). To examine the specificity of these protein-DNA interactions, we conducted a series of preliminary experiments using extracts prepared from HSV-infected cells as a source of endogenous ICP4, radiolabeled DNA fragments from HSV DNA propagated in the vector pBR322 as target DNAs, polyclonal antibody specific for type 1 HSV ICP4 as the immunoprecipitating reagent (36), and a modified DNA binding immunoassay (32). During these initial experiments we observed

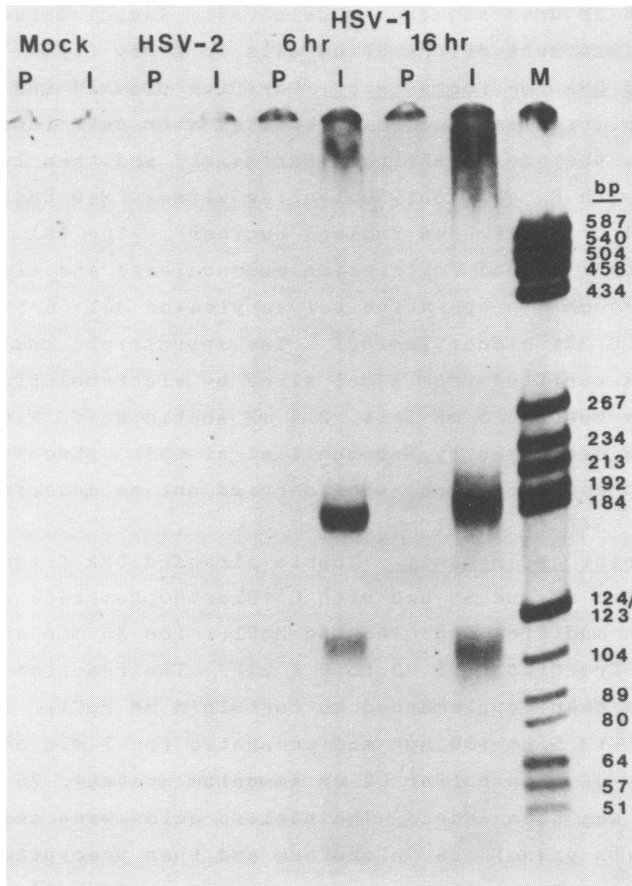


Figure 1. Association of specific DNA fragments with ICP4 in a crude extract. Extracts were prepared at 6 hrs postinfection from HSV-2(G) infected Vero cells, at both 6 and 16 hrs postinfection from HSV-1(HFEM) infected Vero cells, and from uninfected (mock) Vero cells. For each DNA binding immunoassay, 4 μ l of extract was incubated for 30 min at 22°C with 50 ng of a HaeIII digest of radiolabeled pBR322 DNA and 1 μ g of salmon sperm DNA in a total volume of 50 μ l. Preimmune (P) or immune (I) antibody was then added. The immunoprecipitated DNA was analysed by electrophoresis through a 5% polyacrylamide gel and detected by autoradiography, as shown here. The marker (M) lane contains the DNA fragments in a HaeIII digest of radiolabeled pBR322 DNA.

that specific DNA fragments derived from the vector were immunoprecipitated. These observations suggested that stable protein-DNA complexes containing ICP4 and specific nucleotide sequences derived from pBR322 were formed in vitro.

To further investigate this finding, we conducted the DNA binding immunoassay with a HaeIII digest of uniformly radiolabeled pBR322 DNA and crude extracts from mock or HSV-infected Vero cells. A sample of the extract was incubated with both radiolabeled (target) DNA and nonlabeled (carrier) salmon sperm DNA. The radiolabeled DNA fragments associated with ICP4 were immunoprecipitated and analyzed by gel electrophoresis. Two DNA fragments from pBR322 were immunoprecipitated when incubated with extracts from HSV-1(HFEM) infected Vero cells (Fig. 1). Although somewhat degraded by endogenous nucleases, these fragments correspond to the 192 and either the 124 or 123 base pair (bp) fragments in the marker lane. In contrast, no radiolabeled DNA fragments were immunoprecipitated when extracts prepared from uninfected or HSV-2(G) infected Vero cells were used (Fig. 1).

These results demonstrate that specific protein-DNA complexes containing ICP4 were formed in vitro. Furthermore, it is apparent that the factors required for formation of these ICP4-associated protein-DNA complexes are present during both the early (6 hr) and late (16 hr) phases of HSV-1 infection. The negative result with the uninfected cell extract demonstrates that the immunoprecipitation of DNA fragments observed with the infected cell extracts cannot be attributed to a direct recognition by the antibody of a cellular protein which is bound to DNA. This agrees with our previous finding that this preparation of anti-ICP4 antibody is specific for type 1 ICP4 (36).

In order to map the ICP4/protein binding sites in pBR322 more precisely, it was necessary to remove contaminating nucleases and phosphatases from the crude extract. A nuclear extract prepared from Vero cells infected with HSV-1(HFEM) was chromatographed through a molecular sieve matrix as previously described (23). The ICP4 which eluted from the column was further purified by DEAE-cellulose column chromatography, yielding a preparation of ICP4 designated fraction VIII. In comparison to the crude cell extract, fraction VIII had greatly reduced levels of nuclease and phosphatase activity and was approximately 100-fold enriched for ICP4 (data not shown).

When a HaeIII digest of pBR322 was used as target DNA to determine the DNA binding specificity of the ICP4-associated

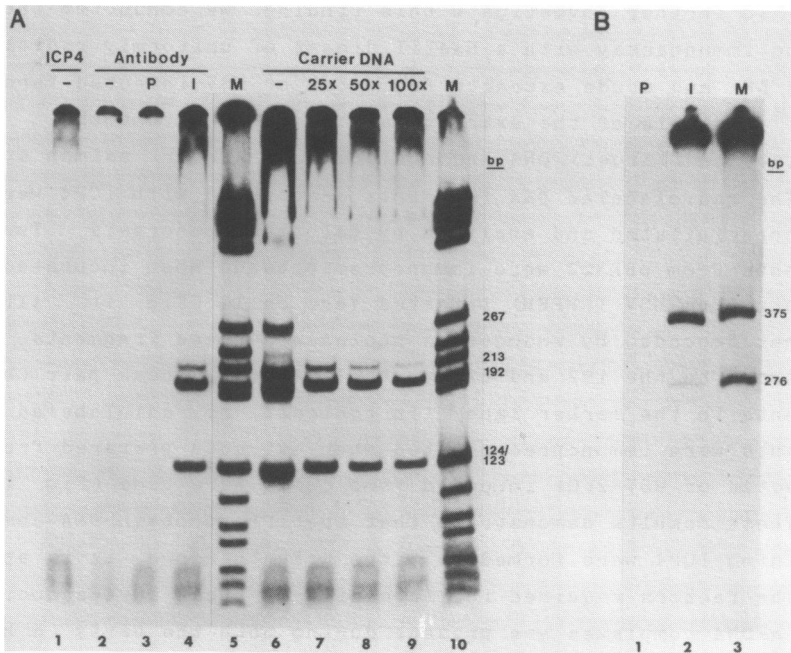


Figure 2. Association of specific DNA fragments with ICP4 in fraction VIII. DNA binding immunoassays (50 μ l total volume) were performed under standard conditions with fraction VIII (0.4 μ l), radiolabeled pBR322 DNA (50 ng), salmon sperm DNA (1.0 μ g), and immune (I) antibody except as indicated. Immunoprecipitated DNA was electrophoresed through a 5% polyacrylamide gel and detected by autoradiography, as shown here. Panel A: The target DNA was a HaeIII digest of pBR322, as shown in the marker (M) lanes. Lane 1- fraction VIII omitted; lane 2- antibody omitted; lane 3- preimmune (P) antibody; lane 6- carrier DNA omitted. For lanes 7-9, the mass ratio of carrier DNA to target DNA is indicated. Panel B: The target DNA was an EcoRI, BamHI, Sall triple digest of pBR322 as shown in the marker (M) lane. P, preimmune antibody; I, immune antibody.

proteins in fraction VIII, we obtained results similar to those obtained with the crude infected-cell extract. In the absence of carrier DNA, four HaeIII fragments corresponding in size to 267, 213, 192, and either 123 or 124 (bp) were associated with ICP4 (Fig. 2A, lane 6). Binding of ICP4-associated proteins to the 267 bp fragment was not detectable in the presence of carrier DNA. Carrier DNA greatly reduced binding of ICP4-associated proteins to the 213 bp fragment (Fig. 2A, lane 7-9). In contrast, binding of

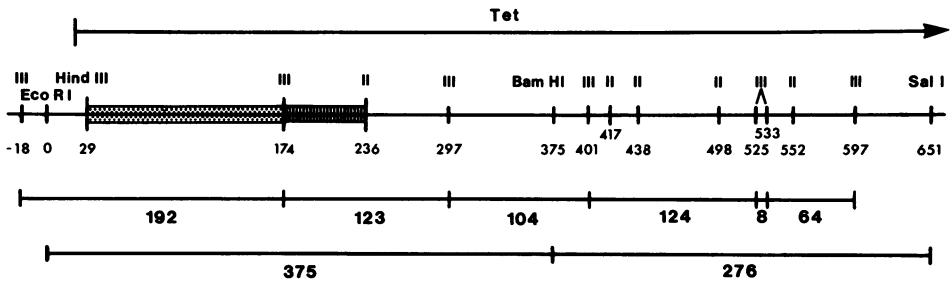


Figure 3. Map of restriction enzyme cleavage sites within the *tet* gene of pBR322. Shown on the second line is a map of a portion of pBR322 spanning nucleotides -18 to +651, where +1 corresponds to the EcoRI cleavage site. The sites and locations of fragments generated by cleavage of this region with HaeIII or with EcoRI plus BamHI plus SalI are indicated on the third and fourth lines, respectively. There are two strong ICP4/protein binding sites within this segment; one (strong site #1) lies within the area enclosed by a stippled box; the other (strong site #2) lies within the area enclosed by a striped box. II = HaeII; III = HaeIII.

ICP4-associated proteins to the 123 (or 124) and 192 bp fragments was only slightly diminished by the addition of a 100-fold excess (by mass) of carrier DNA to the binding reaction (Fig. 2A, lane 9). We conclude that the 192 and either the 123 or the 124 bp HaeIII fragments contain strong ICP4/protein binding sites and that the 213 and 267 bp fragments contain weak and very weak ICP4/protein binding sites, respectively.

In order to test binding of ICP4-associated proteins to either the 123 or the 124 bp HaeIII fragment, we prepared a triple digest of pBR322 DNA with EcoRI, BamHI, and SalI to obtain (i) a 276 bp fragment that contains the 124 bp HaeIII fragment, (ii) a 375 bp fragment that contains the 123 bp HaeIII fragment, and (iii) a 3,712 bp fragment that contains the remainder of the plasmid (Fig. 3). When these fragments were used in the DNA binding immunoassay, the 375 bp fragment was efficiently immunoprecipitated whereas the 275 bp fragment was barely detectable (Fig. 2B, lane 2), indicating that the strong ICP4/protein binding site is located within the 123 bp HaeIII fragment.

The 192 and 123 bp HaeIII fragments are located adjacent to each other and span the 5' end of the gene encoding tetracycline resistance (*tet*) in pBR322 (Fig. 3). Using HindIII, HaeII, and

Hae III digests of the tet gene as target DNAs in additional DNA binding immunoassays (data not shown), we mapped the two strong protein binding sites in pBR322 to locations between nucleotides 29 and 174 and between nucleotides 175 and 236, relative to the EcoRI site in pBR322 (37).

Nucleotide sequence protected by ICP4-associated proteins.

The DNase I footprinting technique (38) was used to identify which regions between nucleotides 29 and 236 in pBR322 form stable associations with proteins in fraction VIII. A 346 bp fragment derived from pBR322 by cleavage with BamHI and HindIII and labeled with ^{32}P at the 5' end of the HindIII site was incubated with samples of fraction VIII and then briefly exposed to DNase I. The resultant radiolabeled DNA fragments were resolved by electrophoresis through an acrylamide-urea gel and detected by autoradiography. In the absence of fraction VIII, a ladder of bands was generated in which the intensity of the bands reflected the relative preference of DNase I for particular cleavage sites in the DNA (39). In the presence of fraction VIII, three distinct interruptions in the ladder were observed (Fig. 4A, lanes 4 - 8). The three protected regions were located within nucleotides 105 to 137, 151 to 166, and 187 to 226. When the experiment was repeated using the same DNA fragment labeled with ^{32}P at the 5' end of the BamHI site, three protected regions corresponding to nucleotides 100 to 138, 148 to 165, and 182 to 223 were observed (Fig. 4B, lanes 4-8).

Because of similarities in size and sequence (see figure 6), we presume that the protected regions between nucleotides 100 and 140 (tet gene strong site #1) and between nucleotides 182 to 226 (tet gene strong site #2) correspond to the ICP4/protein binding sites in the 192 and 123 bp Hae III fragments of pBR322, respectively. The protected region between nucleotides 150 to 166 represents either (i) a third ICP4/protein binding site, (ii) a region protected by a separate protein in fraction VIII, or (iii) a region which is protected by interaction with the ICP4-associated proteins bound at the flanking sites.

Association of ICP4 with specific nucleotides in HSV-1 DNA.

In preliminary DNA binding immunoassays with restriction enzyme digests of genomic HSV-1 DNA and fraction VIII, numerous viral DNA

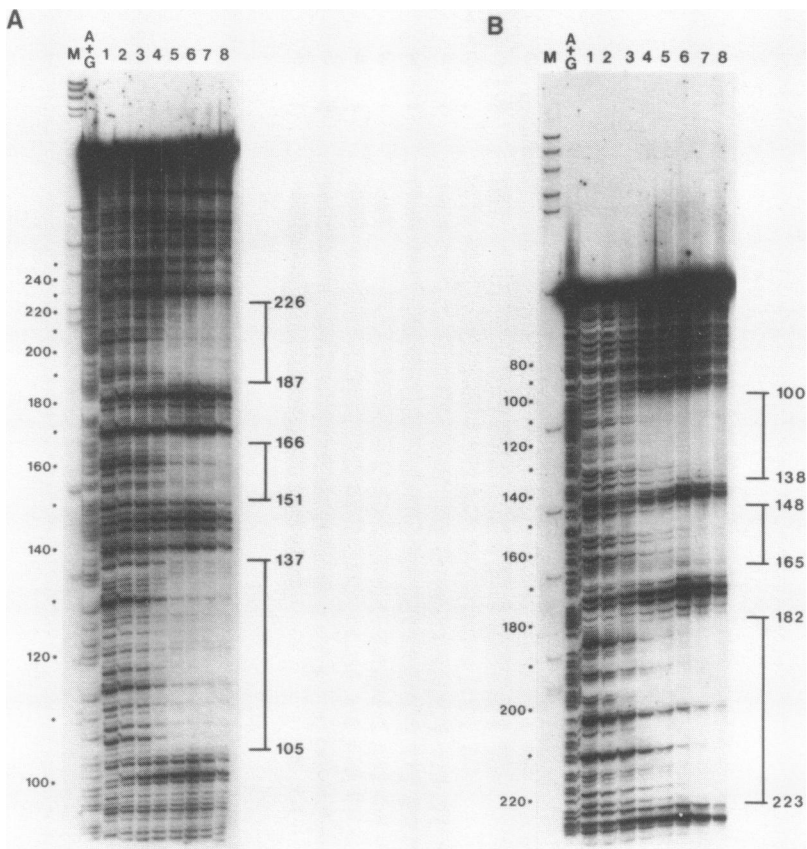


Figure 4. DNase I footprint of ICP4/protein binding sites in the *tet* gene. The 346 bp HindIII-BamHI fragment from pBR322, labeled at one 5' terminus with ^{32}P , was incubated with fraction VIII and then treated with DNase I. Radiolabeled digestion products were separated by electrophoresis through an 8% polyacrylamide gel in the presence of 8.3M urea and detected by autoradiography. Panel A: DNA (20 ng) labeled at the 5' terminal nucleotide of the HindIII site was incubated with fraction VIII (lanes 1-8: 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, or 6.4 μl , respectively) and DNase I (lanes 1-8: 12, 12, 15, 18, 25, 40, 65, or 100 ng, respectively). Panel B: DNA (20 ng) labeled at the 5' terminal nucleotide of the BamHI site was incubated with the same amounts of fraction VIII and DNase I as in Panel A. Lane M- HaeIII digest of pBR322 DNA; Lane A+G- DNA fragments produced by partial cleavage of the end-labeled fragment at adenines and guanines (34). The numbers on the left and right sides of each panel indicate the position of the nucleotide at the 3' end of each radiolabeled fragment, where position +1 corresponds to the EcoRI site in pBR322.

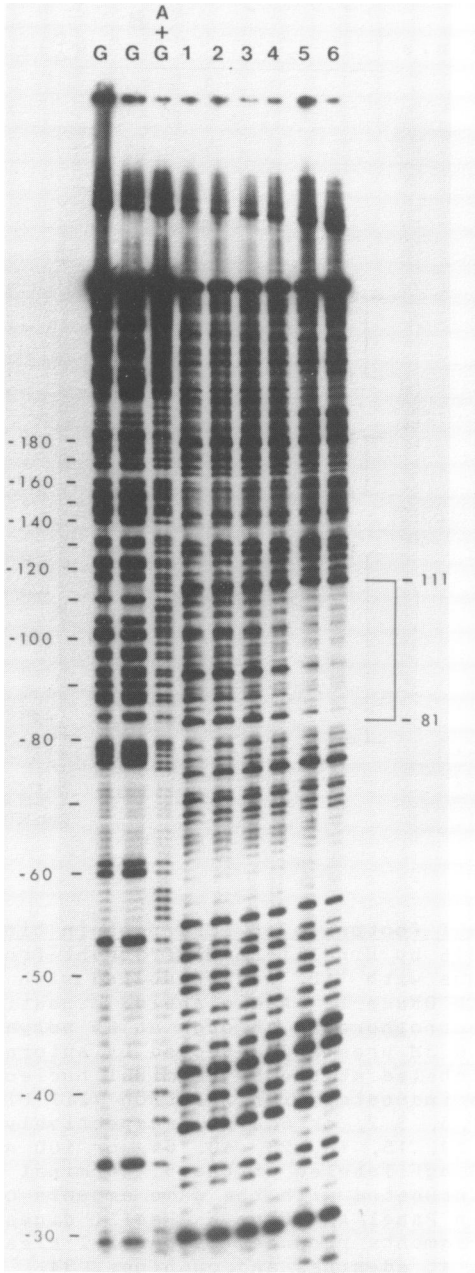


Figure 5. DNase I footprint of ICP4/protein binding site in the HSV gD gene. A 403 bp fragment (approximately 20 ng) extending from a HindIII site at +11 to an SstI site at -392 relative to the

matches, and only 3 complete mismatches. Of particular interest are the sequences ATCGTC and YCGRC (Y = pyrimidine, R = purine) which are present at the same locations in all three sites and thus may form part of the recognition sequence required for binding of ICP4-associated proteins to DNA.

DISCUSSION

The results from the DNA binding immunoassays presented in this report demonstrate that ICP4 can associate with a stable protein-DNA complex. The results also indicate that the protein component(s) of the complex bind(s) only to specific nucleotide sequences. The results do not reveal whether the nucleotide-sequence specificity of the protein-DNA complex is determined by ICP4 itself, by another protein in fraction VIII, or by a complex between ICP4 and another protein. Pertinent to this discussion is a description by Freeman and Powell (24) of a partially purified preparation of ICP4 that did not bind to single-stranded salmon sperm DNA unless the preparation was supplemented with a crude extract from uninfected cells. Their results suggest that ICP4 binds to single-stranded DNA via a host cell component. Additional experiments are required to determine if the mechanism for nonspecific association between ICP4 and single-stranded DNA described by Freeman and Powell is related to the mechanism for sequence-specific association between ICP4 and double-stranded DNA described in this report and by Kristie and Roizman (24a).

The presence of two strong ICP4/protein binding sites within the well-defined, fully sequenced pBR322 plasmid provided an opportunity to compare sequences for two independent binding sites with each other and with sequences in the remaining 4,300 bp of BR322 to which ICP4 bound weakly or not at all. A comparison of the sequences within the three ICP4/protein binding sites reported in this paper reveals certain similarities which presumably constitute part of the recognition site for a sequence-specific binding protein in fraction VIII. The sequence 5'-ATCGTC-3' is present in all three sites. In addition to the two strong binding sites, there are four other regions where the sequence 5'-ATCGTC-3' occurs in pBR322. These four sites are located in three different HaeIII fragments that are 434, 234(two sites), and 213

bp in size. Of these three fragments, the 213 bp fragment exhibited a weak association with ICP4 (fig. 2). The relatively weak binding to the 213 bp fragment and the absence of the 434 and 234 bp fragments in the immunoprecipitate suggest that definition of the binding site requires more than 6 nucleotides. Examination of sequence similarities in the binding sites (fig. 6) suggests that the DNA recognition site may consist of clusters of 5 or 6 essential nucleotides located at equal intervals along 3 or 4 turns of the DNA helix such that all clusters are located on one "face" of the helix. We tentatively propose that the first two clusters are defined by the sequences ATCGTC and YCGRC (Y = pyrimidine; R = purine) and that the interval between these clusters is 4 nucleotides. A search of HSV sequences with the probe 5'-ATCGTCN₄YCCGRC-3' revealed closely-related sequences in the promoters for the IE genes encoding ICP4 and ICP0; no related sequence was found in the promoters for the genes encoding the other IE early genes or in the promoter for the thymidine kinase gene. These findings agree with our binding studies conducted with these promoters (Faber and Wilcox, in preparation) and, with the exception of the ICP27 gene promoter, with those of Kristie and Roizman (24a).

We have demonstrated that there are ICP4/protein binding sites in HSV-1 DNA and that one of these sites is located approximately 100 nucleotides upstream from the transcription start site of an HSV early gene. However, these findings represent only circumstantial evidence that specific interactions between ICP4 and the viral genome influence transcription of viral genes in vivo. In a more direct approach, Beard et al (41) observed that addition of fraction VIII to an in vitro transcription system stimulated transcription from the gD promoter. Experiments are in progress to determine what role the ICP4/protein binding site at position -100 in the gD gene plays in this observed stimulation. This point is particularly significant because Everett, using an in vivo approach, reported that "all the sequence elements required for regulated expression of the gD gene lie within 83 bp of the RNA capsites" (42). Everett also reported that within this 83 bp region, no "sequence elements involved solely and specifically in trans activation ... were detected"

(5). Thus it has been proposed that the regulation of transcription by ICP4 is not mediated by a specific interaction between ICP4 and viral DNA and may instead be mediated by interaction with a cellular factor, as has been proposed for the E1a gene product of adenovirus (43,44) and the IE (immediate early) protein of pseudorabies virus (45). Further investigations with purified preparations of these proteins are needed to define the interactions between viral proteins, cellular transcription factors, and viral promoters that are required for regulated expression of genes in the eucaryotic cell nucleus.

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