Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4700–4704, July 1986
Biochemistry

DNA-binding site of major regulatory protein α4 specifically associated with promoter–regulatory domains of α genes of herpes simplex virus type 1

(DNA–protein complexes/gel electrophoresis assay/exonuclease III/monoclonal antibody)

THOMAS M. KRISTIE AND BERNARD ROIZMAN

The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, 910 East 58th Street, Chicago, IL 60637

Contributed by Bernard Roizman, March 13, 1986

ABSTRACT **Herpes simplex virus type 1 genes form at least five groups (α, β_1, β_2, γ_1, and γ_2) whose expression is coordinately regulated and sequentially ordered in a cascade fashion. Previous studies have shown that functional α4 gene product is essential for the transition from α to β protein synthesis and have suggested that α4 gene expression is autoregulatory. We have previously reported that labeled DNA fragments containing promoter–regulatory domains of three α (α0, α4, and α27) and a γ_2 gene form stable complexes with proteins from lysates of infected cells as detected by a gel electrophoresis binding assay and that monoclonal antibody to α4 protein reduced the electrophoretic mobility of the complex of labeled DNA and protein from infected cells. In this study we identified one monoclonal antibody, H950, from a panel of monoclonal antibodies to the α4 protein that blocks the formation of specific infected cell complexes with labeled DNA fragments containing promoter and regulatory domains of α genes. We also report the nucleotide sequence of the α0 promoter domain protected from exonuclease III digestion by α4 protein in the absence of H950 monoclonal antibody but not in its presence. In addition, we identified a 59-base-pair sequence from the regulatory domain of the α4 gene that binds α4 protein. Deletion clones of this fragment localize sequence elements required for formation of the α4 protein–DNA complex. Furthermore, deletion of the *in vitro* binding site of the SP1 transcription factor from the 59-base-pair fragment did not affect the formation of the α4 protein–DNA complex.**

The herpes simplex virus type 1 (HSV-1) genes form several groups (α, β_1, β_2, γ_1, and γ_2) whose expression is coordinately regulated and sequentially ordered in a cascade fashion (1). The five α genes (α0, α4, α22, α27, and α47) are the first viral genes to be transcribed after infection. Functional α proteins, especially α4, are required for the expression of β and γ genes (ref. 2, reviewed in ref. 3). α22 and α27 appear to be required for optimal expression of the later groups of genes (4, 5). The two γ groups, γ_1 and γ_2, can be differentiated by the dependence of γ_2 genes upon DNA synthesis for their expression (3).

The focus of our interest is on the function and regulation of α gene expression. α gene expression is enabled by at least two sets of factors. In the promoter–regulatory region of α genes, there are G+C-rich elements that respond to both host and viral factors (6, 7) and specific A+T-rich elements that respond to *trans*-acting factors packaged in the virion (6, 8–10). The gene specifying the α-*trans*-inducing factor (αTIF) has been sequenced (11, 12) but its mechanism of action is not known. Numerous studies have shown that α gene expression is turned off by viral proteins made later in infection (1, 13, 14) and probably also by the product of the α4 gene (2, 15, 16).

Earlier we reported that labeled HSV-1 DNA fragments containing promoter and regulatory domains of α, β, and γ genes specifically bind infected cell proteins (17), as detected by retardation of the electrophoretic mobility of the DNA in nondenaturing polyacrylamide gels (18–20). We have also shown that these complexes contain the α4 protein inasmuch as monoclonal antibody to the α4 protein retards the electrophoretic mobility of the DNA–protein complex, whereas monoclonal antibodies to several other HSV-1 α proteins had no effect (17). In this paper we report on the DNA-binding sites of the infected cell protein complex containing the α4 protein.

MATERIALS AND METHODS

Cells, Virus, and Protein Extracts. HeLa cells, grown to confluence in 150-cm^2 flasks, were mock infected or infected with 5 plaque-forming units of HSV-1 strain F [HSV-1(F)] (21) and maintained for 10 hr. Nuclear extracts (5–7 mg of protein per ml) were prepared as described except that Nonidet P-40, at a concentration of 0.025%, was included in the nuclear wash prior to protein extraction (22, 23).

Cloning and Preparation of DNA Probes. The DNA fragments shown in Fig. 1 were cloned by standard techniques (28) and sequenced by the dideoxynucleotide technique (29). The DNA fragment probes were 5′-end-labeled with [γ-^{32}P]ATP as described (28, 30) to an activity of 10,000 to 15,000 cpm/ng of DNA fragment.

Protein–DNA Binding Assays. Unless otherwise stated, protein–DNA binding assays were done as described (17) and as summarized in the legend to Fig. 2.

Exonuclease III Digestions. Plasmid DNA carrying the appropriate viral fragment was digested with *Hin*dIII or *Eco*RI, dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim), 5′-end-labeled with [γ-^{32}P]ATP (>7000 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) by T4 polynucleotide kinase (United States Biochemical, Cleveland, OH), and digested with *Eco*RI or *Hin*dIII, respectively. The resulting fragment labeled at one terminus was extracted from polyacrylamide gels and purified as described (30). Then 10,000 cpm (approximately 2 ng of DNA fragment) was incubated for 0.5 hr in the DNA-binding reaction in the presence of 2000 ng of poly(dI)·poly(dC) as described (17). After incubation, the mixture was adjusted to 2.5 mM $MgCl_2$ and exonuclease III (New England Biolabs) was added as detailed in the legend to Fig. 4. The reaction was terminated by the addition of sodium dodecyl sulfate and EDTA (0.1% and 10 mM final concentrations). The digested

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HSV-1, herpes simplex virus type 1; TK, thymidine kinase; bp, base pair(s).

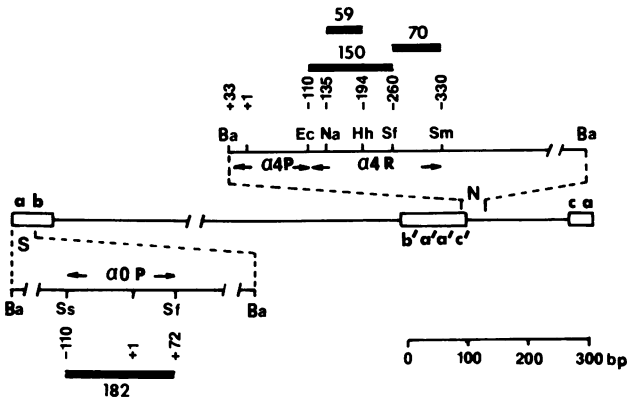

FIG. 1. Sequence arrangement of HSV-1 DNA and locations of DNA fragments tested. The boxed terminal sequences *ab* and *ca* are repeated internally as the inverted sequences *b'a'a'c'* (24). Expanded scales show the location of the *α0* promoter domain (*α0P*) and the *α4* promoter (*α4P*) and regulatory (*α4R*) domains (6, 25, 26). The thick lines designated by base pair (bp) length represent the cloned fragments tested in these studies. These were the 182-bp *Sst* II–*Sfa*NI fragment of *Bam*HI fragment S cloned in the *Pst* I–*Sma* I site of pUC9 (27) as pRB3563; the 150-bp *Eco*RI–*Sfa*NI fragment of *Bam*HI fragment N cloned in the *Sma* I site of pUC9 as pRB3431; the 70-bp *Sfa*NI–*Sma* I fragment of *Bam*HI fragment N cloned in the *Sma* I site of pUC9 as pRB3430; and the 59-bp *Nae* I–*Hha* I fragment of *Bam*HI fragment N cloned in the *Hin*cII site of pUC9 as pRB3085 (7). Restriction endonuclease sites: Ba, *Bam*HI; Ss, *Sst* II; Sf, *Sfa*NI; Ec, *Eco*RI; Na, *Nae* I; Hh, *Hha* I; Sm, *Sma* I.

DNA was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol) and once with chloroform/isoamyl alcohol (24:1) and precipitated with ethanol. The radioactivities were measured, the samples were resuspended in 20 mM EDTA/0.1% xylene cyanol/92% (vol/vol) formamide and denatured at 80°C, and 1000 cpm was separated on a 6% polyacrylamide sequencing gel as described (30).

Monoclonal Antibodies to α4. The monoclonal antibodies H640, H953, H1091, H948, H943, H942, H949, H950, H924, and H944, in the form of ascites fluids, were a gift of Lenore Pereira and were in part described elsewhere (31).

RESULTS

Detection of α4 Protein–α0 Promoter DNA Complexes with a Panel of Anti-α4 Monoclonal Antibodies. Previously we reported that proteins in lysates of cells infected with HSV-1 form specific complexes with short DNA sequences containing promoter–regulatory domains of HSV-1 genes. These complexes are readily detected as a consequence of a reduction of the electrophoretic mobility of the labeled DNA fragments in nondenaturing polyacrylamide gels. We also reported that monoclonal antibody to α4 protein, but not to other α proteins, further reduced the electrophoretic mobility of the DNA–protein complexes, indicating that the complexes contained α4 protein (17). In this section we report, as illustrated in Fig. 2, that most of the 10 independently derived monoclonal antibodies to α4 tested (e.g., H640, H953, H1091, H948, H943, H949, H924, H942, and H944) reduced the electrophoretic mobility of labeled DNA–infected cell protein complexes when antibody was added after protein–DNA binding without significantly affecting the formation of the α4 protein–DNA complex. The labeled DNA fragment tested contained the *α0* promoter domain (Fig. 1, 182 DNA) and the results indicated that binding of many of the monoclonal antibodies did not alter the affinity of the α4 protein for this DNA. The exceptions are exemplified by H942, which reduced the formation of the protein–DNA complex when

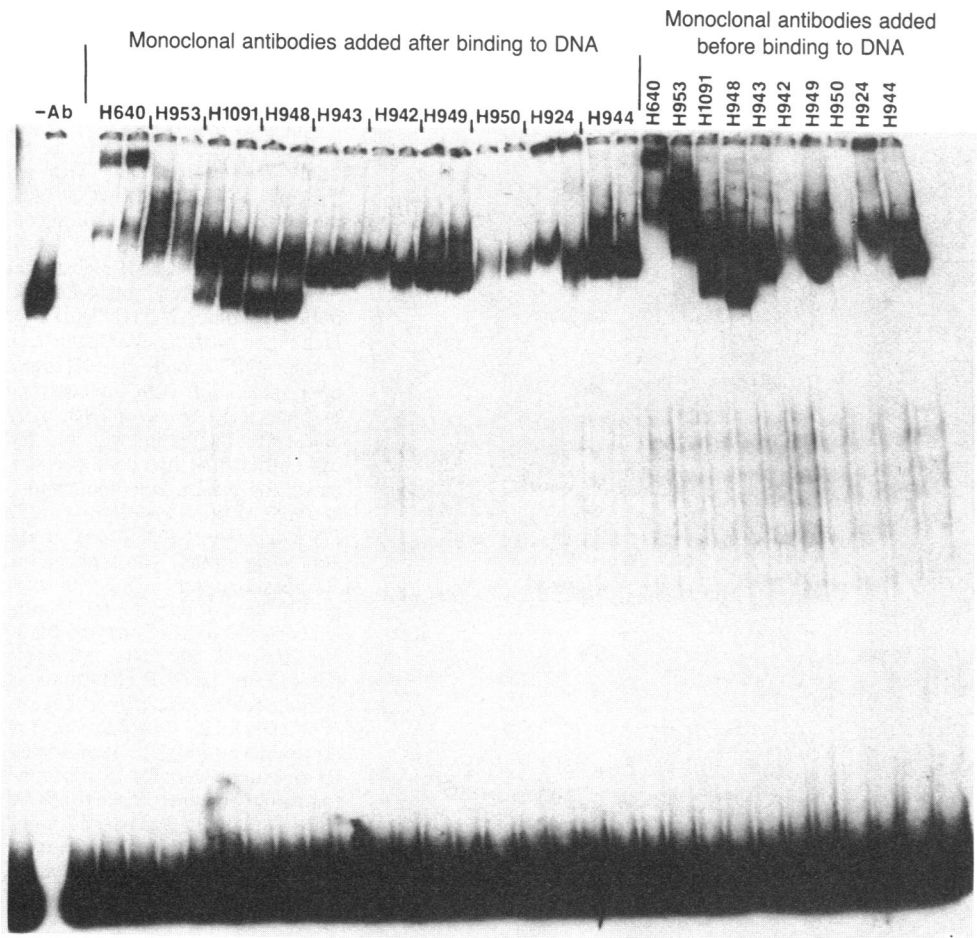

FIG. 2. Autoradiographic image of labeled 182 DNA complexed with proteins from HSV-1-infected cells and exposed to a panel of monoclonal antibodies against α4 protein. DNA–protein binding reactions were as described (17). Labeled 182 DNA (2.5 ng) and 1500 ng of competitor nucleic acid poly(dI)·poly(dC) (Pharmacia P-L Biochemicals) were incubated with 1000 ng of HSV-1-infected cell protein in 20 mM Tris·HCl, pH 7.6/50 mM KCl/0.05% Nonidet P-40/5% (vol/vol) glycerol, 50 μg of bovine serum albumin (Sigma) per ml/10 mM 2-mercaptoethanol/1 mM EDTA for 0.5 hr. The indicated anti-α4 monoclonal antibody either was added after protein–DNA binding or was incubated with the protein extract for 0.5 hr prior to the addition of the extract to the binding reaction mixture for an additional 0.5 hr. The −Ab lane contains 182 DNA–protein complexes formed in the absence of added monoclonal antibody. The mixture in the first lane of each pair contained 1000 ng of murine ascites fluid protein, and the mixture in the second lane contained 500 ng. Preincubations were done with 1000 ng of monoclonal antibody per 1000 ng of protein from HSV-1-infected cells.

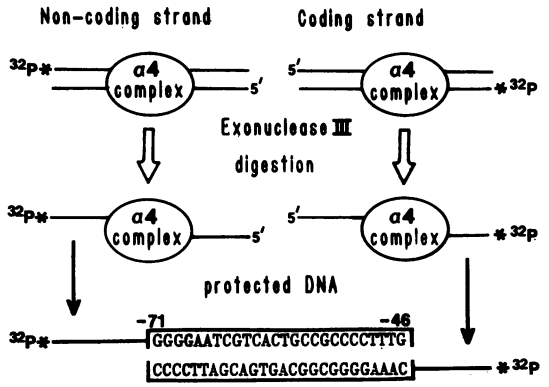

FIG. 3. Schematic representation of an exonuclease III protection experiment and the location of the $\alpha 0$ promoter (182 DNA) fragment sequences protected by $\alpha 4$ protein. Noncoding or coding strand refers to the strand 5'-end-labeled with [γ-^{32}P]ATP. The overlapping nucleotide sequences (−71 to −46) of 182 DNA protected from digestion by $\alpha 4$ protein were numbered relative to the transcription initiation site of the $\alpha 0$ mRNA (6, 25).

added to the extract before incubation with labeled DNA, and H950, which reduced the formation of the complex when added either before or after protein–DNA binding. The characteristic pattern obtained with each monoclonal antibody suggests that the antibodies were directed against different epitopes of the $\alpha 4$ protein and are thus useful tools for analyses of the domains of the $\alpha 4$ protein.

Mapping of the Binding Site of the $\alpha 4$ Protein to $\alpha 0$ Promoter DNA. As illustrated in Fig. 3, DNA fragments labeled with ^{32}P at one 5' terminus and specifically bound to infected cell proteins were digested with exonuclease III. The DNA protected from digestion was sized on sequencing gels and the nucleotide sequence bound by the protein complex emerged from the comparison of the 3' boundaries of the protected coding and noncoding strands. Exonuclease III processively digests double-stranded DNA from recessed 3' termini (32), but the exonucleolytic activity is blocked by proteins bound to the DNA. The enzyme has been used to detect protein–DNA complexes *in vitro* and *in vivo* and is well suited for mapping complexes in crude nuclear extracts (33–35).

Fig. 4 *Left* shows the results of exonuclease III digestion of $\alpha 0$ promoter DNA (Fig. 1, 182 DNA), labeled at the 5' end of the noncoding strand. The $\alpha 0$ promoter DNA was incubated without extract, with mock-infected cell extract, with infected cell extract, or with infected cell extract that had been preincubated with anti-$\alpha 4$ antibody H950, which, as shown in the preceding section, significantly inhibited the binding of $\alpha 4$ protein to the $\alpha 0$ promoter DNA. With increasing time of digestion, a unique 63-nucleotide fragment becomes progressively evident in the infected extract reaction. In contrast, no unique DNA fragment incubated without extract or with mock-infected extract was protected from exonucleolytic digestion. DNA digested in the presence of infected cell extract, preincubated with H950, showed a substantial reduction in the protection of the 69-nucleotide fragment. A similar digestion of $\alpha 0$ promoter DNA labeled at

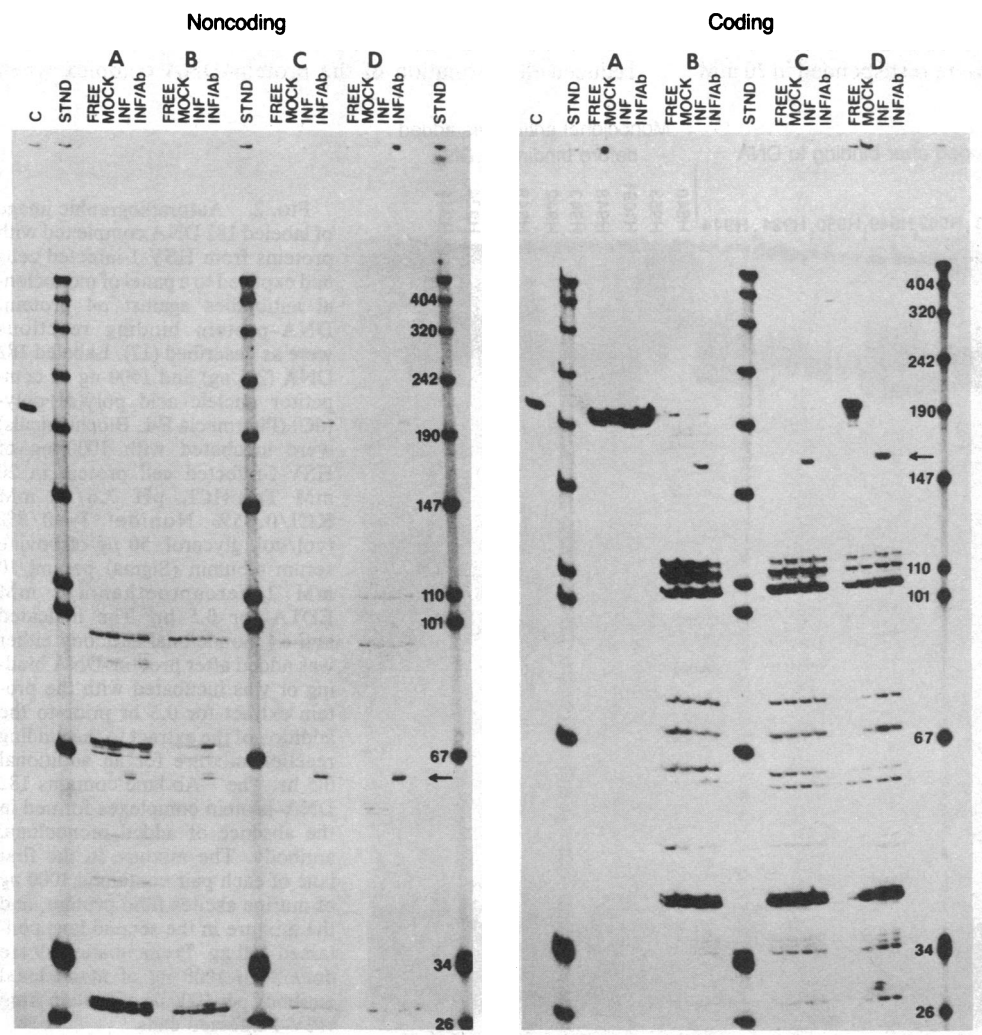

FIG. 4. Autoradiogram of electrophoretically separated exonuclease III digests of 182 DNA in the absence or presence of available $\alpha 4$ protein. Fragments of pUC9 (27) digested with *Hpa* II restriction enzyme and end-labeled with [γ-^{32}P]ATP (STND) served as size markers; sizes are given in bp. Lanes: C, nondigested 182 DNA; FREE, exonuclease III digest after incubation without protein extract; MOCK, exonuclease III digest after incubation with mock-infected cell extract; INF, exonuclease III digest after incubation with infected cell extract; INF/Ab, exonuclease III digest after incubation with infected cell extract that had been preincubated for 0.5 hr with monoclonal antibody H950. (*Left*) Digests of 182 DNA labeled at the 5' end of the noncoding strand. The exonuclease III digestions were as follows: lanes A, 100 units of enzyme for 15 min; lanes B, 100 units of enzyme for 30 min; lanes C, 100 units of enzyme for 45 min; lanes D, 200 units of enzyme for 30 min. (*Right*) Digests of 182 DNA labeled at the 5' end of the coding strand. The exonuclease III digestions were for 15 min at the following enzyme concentrations: lanes A, 1 unit; lanes B, 25 units; lanes C, 50 units; lanes D, 100 units. The arrows indicate the positions of bands representing DNA protected by the $\alpha 4$ protein.

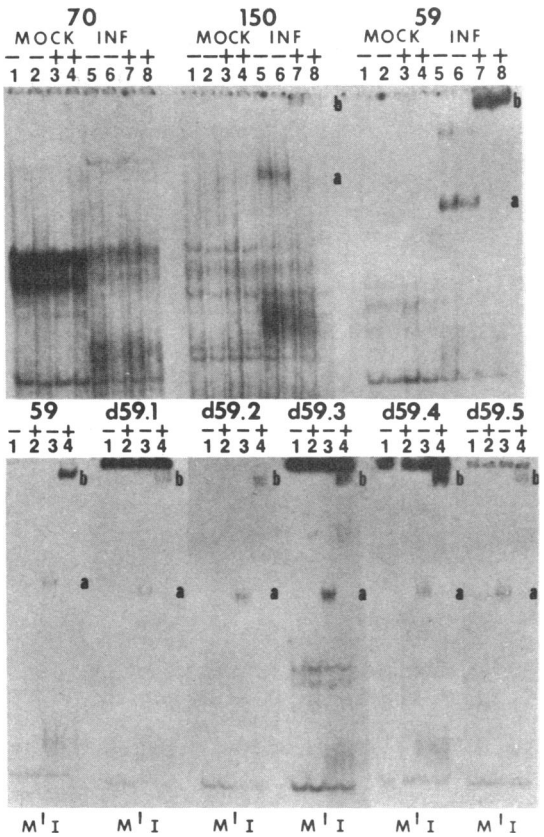

FIG. 5. Localization of the α4 protein DNA-binding site within the *α4* gene regulatory domain. (*Upper*) Autoradiographic images of labeled DNA fragments, subcloned from the *α4* regulatory domain (6, 26), reacted with proteins from mock-infected cells (lanes 1–4) or infected cells (lanes 5–8) and incubated in the presence or absence of anti-α4 monoclonal antibody. The left, center, and right gels represent the 70-, 150-, and 59-bp α4R subclones, respectively. The map positions of the subcloned fragments are shown in Fig. 1. Mixtures in lanes 1, 3, 5, and 7 contained 1000 ng of competitor poly(dI)·poly(dC), whereas those in lanes 2, 4, 6, and 8 contained 1500 ng of competitor. −, No monoclonal antibody added; +, 1000 ng of anti-α4 monoclonal antibody H640. (*Lower*) Autoradiographic images of labeled wild-type 59-bp α4R subclone and the deletion clones of this sequence (Fig. 6) complexed with proteins of mock-infected or infected cells and incubated in the presence or absence of anti-α4 monoclonal antibody. Mixtures shown in lanes 1 and 2 contained mock-infected cell extracts (M) whereas those in lanes 3 and 4 contained infected cell extracts (I). All reaction mixtures contained 1500 ng of competitor poly(dI)·poly(dC). −, No monoclonal antibody added; +, 1000 ng of anti-α4 monoclonal antibody H640.

the 5′ end of the coding strand is shown in Fig. 4 *Right*. In this case, a unique protected DNA fragment 163 nucleotides long emerged in exonuclease III digests of mixtures containing infected cell proteins. The fragment was not protected from exonucleolytic digestion in mixtures lacking infected cell proteins and was substantially reduced in mixtures containing infected cell proteins preincubated with antibody H950. The noncoding and coding strand protected share the 26-nucleotide sequence represented in Fig. 3 as position −46 to −71 relative to the transcription initiation site of *α0* mRNA (6, 25).

Location of the Binding Site of α4 Protein Complex to Its Own Regulatory Domain. We have previously reported that α4 protein is present in specific infected cell protein–DNA complexes containing promoter–regulatory domains of α genes, including the regulatory domain of the *α4* gene. To localize the sequences required for this complex formation, the *α4* regulatory domain, which extends from −110 to −330 relative to the *α4* mRNA transcription initiation site (26), was subcloned as shown in Fig. 1. Fig. 5 *Upper* shows the binding of the 70-, 150-, and 59-bp subclones in mock-infected or infected cell extracts and in the presence or absence of monoclonal antibody to the α4 protein. The α4 protein is readily apparent in complex a formed by the 143- and 59-bp fragments in the absence of anti-α4 monoclonal antibody and by the shift in mobility to position b in the presence of antibody. In contrast, a complex of α4 protein and the 70-bp subclone was not readily detected although a host protein–DNA complex was clearly evident. The nature of this host complex requires further investigation. As the 59-bp fragment is contained within the 150-bp fragment (Fig. 1), we constructed the series of deletions shown in Fig. 6. The ability of these deletion fragments to form the α4 protein–DNA complex was tested in the absence or presence of monoclonal antibody to α4 protein. As shown in Fig. 5 *Lower*, all the constructed deletions were competent to form the α4 protein–DNA complex. Since the juxtaposed sequences do not reconstruct the authentic sequence in the deletion constructs, it is likely that the α4 protein-binding site is within the right half of the 59-bp fragment, proximal to the α4 promoter domain. It remains to be determined, however, whether the previously identified *cis*-acting functions of the 59-bp fragment were also conserved in the deletion fragments.

The deletions 59.1–59.3 progressively remove the sequence TGGGCGGGGC, which has been shown to interact with the SP1 factor *in vitro* (36). The removal of this binding site does not appear to affect the efficiency of binding of the α4 protein complex to these DNA fragments.

DISCUSSION

α4, or infected cell polypeptide no. 4 (ICP4) (37), is a homodimer phosphoprotein (38, 39) with a monomeric molecular weight of approximately 160,000 (40). It is the major

```
          -194                                                                -135
59        CGGATGGGCGGGGCCGGGGGTTCGACCAACGGGCCGCGGCCACGGGCCCCCGGCGTGCCG
d59.1       GGATCCGGGCCGGGGGTTCGACCAACGGGCCGCGGCCACGGGCCCCCGGCGTGCCG
d59.2         GGATCCCCGGGGGTTCGACCAACGGGCCGCGGCCACGGGCCCCCGGCGTGCCG
d59.3               GGATCCGGGTTCGACCAACGGGCCGCGGCCACGGGCCCCCGGCGTGCCG
d59.4     CGGATGGGCGGGGCCGGGGGT.....GGCCACGGGCCCCCGGCGTGCCG
d59.5     CGGATGGGCGGGGCCGGGGGT.....CCACGGGCCCCCGGCGTGCCG
```

FIG. 6. Nucleotide sequence of the intact and the deletion 59-bp DNA fragments containing the α4 binding site from the regulatory domain of the *α4* gene. Deletion clones d59.1 (pRB3190), d59.2 (pRB3189), and d59.3 (pRB3191) were constructed by digestion of *Bam*HI-cleaved pRB3085 with BAL-31 and insertion of a *Bam*HI linker at the site of deletion. Deletion clones d59.4 (pRB3187) and d59.5 (pRB3188) were constructed by partial digestion of pRB3085 with *Taq* I in the presence of ethidium bromide followed by complete digestion with *Sst* II and treatment with S1 nuclease. The deletions were verified by sequencing as described (29). The underlined sequence represents the SP1 *in vitro* binding site (36). Boxed sequences represent the *Bam*HI linker sequence. Dotted lines represent the nucleotides deleted from the 59-bp sequence.

regulatory protein encoded by HSV-1, inasmuch as temperature-sensitive lesions in the α4 protein affect not only the transition from α to β and γ protein synthesis but also the expression of α genes (2, 3, 40–42). We have previously shown that α4 binds specifically to promoter–regulatory domains of α, β, and γ genes in crude cell extracts (17), using the gel electrophoresis retardation assay (18–20). The binding of α4 protein in infected cell extracts to labeled promoter and regulatory DNA fragments was not affected by competition with salmon sperm DNA, competitor polydeoxynucleotide chains, or heterologous DNA fragments. Monoclonal antibody to α4 protein, but not to other viral proteins, further reduced the mobility of the DNA–protein complexes when added to the DNA-binding mixtures.

In this paper we report the nucleotide sequence of the binding site of the α4 protein complex to the promoter domain of the *α0* gene and identify the sequence within the regulatory domain of the *α4* gene that binds the α4 protein. An interesting feature of the located binding sites is their proximity to various host factor binding sites such as CCAAT (43) and SP1 (36). It is conceivable that the proximity of the host components may be critical in both the positive and negative regulatory functions of the α4 protein. As shown in *Results*, however, the binding of α4 does not require conservation of the SP1 binding site.

The identification of the α4 binding site in the 59-bp sequence of the α4 regulatory region is of particular interest in light of the *cis*-acting functions that were identified in earlier studies (7). This sequence element conferred a high constitutive level of expression upon chimeric genes containing the α4 promoter (−110 to +33) or the β-regulated promoter of the thymidine kinase gene but did not confer the capacity to be regulated as an α gene. The higher constitutive level of expression of the chimeric genes may be due to juxtaposition of additional binding sites for host factors—e.g., SP1—rather than for α4 protein inasmuch as the elevated basal level of expression was independent of α4 protein. Second, the fusion of the 59-bp fragment to the β-regulated promoter chimera restored the capacity of this gene to be highly induced by α4 protein. The studies presented in this paper suggest that the 59-bp fragment substituted for elements in the second distal signal, mapped by McKnight (44, 45) in the β-regulated promoter, by providing an α4 binding site.

Numerous studies suggest that α4 is a multifunctional protein. It remains to be seen whether the position or diversity of the binding sites underlies the multifunctionality of the α4 protein.

We thank Lenore Pereira for the monoclonal antibodies and Nancy Michael for excellent technical assistance. These studies were aided by grants from the National Cancer Institute (CA08494 and CA19264) and the American Cancer Society (MV2T). T.K. is a National Cancer Institute Predoctoral Trainee (CA192642).

1. Honess, R. W. & Roizman, B. (1974) *J. Virol.* **14,** 8–19.
2. Watson, R. J. & Clements, J. B. (1980) *Nature (London)* **285,** 329–330.
3. Roizman, B. & Batterson, W. (1985) in *Virology*, ed. Fields, B. (Raven, New York), pp. 497–526.
4. Sears, A. E., Halliburton, I. W., Meignier, B., Silver, S. & Roizman, B. (1985) *J. Virol.* **55,** 338–346.
5. Sacks, W. R., Greene, C. C., Aschman, D. P. & Schaffer, P. A. (1985) *J. Virol.* **55,** 796–805.
6. Mackem, S. & Roizman, B. (1982) *J. Virol.* **44,** 939–949.
7. Kristie, T. M. & Roizman, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4065–4069.
8. Post, L. E., Mackem, S. & Roizman, B. (1981) *Cell* **24,** 555–565.
9. Batterson, W. & Roizman, B. (1983) *J. Virol.* **46,** 371–377.
10. Roizman, B., Kristie, T., Batterson, W. & Mackem, S. (1984) in *Transfer and Expression of Eukaryotic Genes*, eds. Ginsberg, H. S. & Vogel, H. J. (Academic, New York), pp. 227–238.
11. Pellett, P., McKnight, J. L. C., Jenkins, F. & Roizman, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5870–5874.
12. Dalrymple, M. A., McGeoch, D. J., Davison, A. J. & Preston, C. M. (1985) *Nucleic Acids Res.* **13,** 7865–7879.
13. Fenwick, M. & Roizman, B. (1977) *J. Virol.* **22,** 720–725.
14. Read, G. S. & Frenkel, N. (1983) *J. Virol.* **46,** 498–512.
15. Knipe, D. M., Ruyechan, W. T., Roizman, B. & Haliburton, I. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3896–3900.
16. O'Hare, P. & Hayward, G. S. (1985) *J. Virol.* **56,** 723–733.
17. Kristie, T. M. & Roizman, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3218–3222.
18. Fried, M. & Crothers, D. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
19. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
20. Strauss, F. & Varshavsky, A. (1984) *Cell* **37,** 889–901.
21. Ejercito, P. M., Kieff, E. D. & Roizman, B. (1968) *J. Gen. Virol.* **2,** 357–364.
22. Dignam, J. D., Lebovitz, R. M. & Roeder, R. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
23. Enomoto, T., Sei-ichi, T. & Yamada, M. (1983) *Biochemistry* **22,** 1128–1133.
24. Roizman, B. (1979) *Annu. Rev. Genet.* **13,** 25–57.
25. Mackem, S. & Roizman, B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7122–7126.
26. Mackem, S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4917–4921.
27. Vieira, J. & Messing, J. (1982) *Gene* **19,** 259–268.
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
29. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
30. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
31. Ackermann, M., Braun, D. K., Pereira, L. & Roizman, B. (1984) *J. Virol.* **52,** 108–118.
32. Rogers, S. G. & Weiss, B. (1980) *Methods Enzymol.* **65,** 201–210.
33. Wu, C. (1984) *Nature (London)* **309,** 229–234.
34. Wu, C. (1985) *Nature (London)* **317,** 84–87.
35. Kovesdi, I., Reichel, R. & Nevins, J. R. (1986) *Science* **231,** 719–722.
36. Jones, K. A. & Tjian, R. (1985) *Nature (London)* **317,** 179–185.
37. Honess, R. W. & Roizman, B. (1973) *J. Virol.* **12,** 1346–1365.
38. Metzler, D. W. & Wilcox, K. (1985) *J. Virol.* **55,** 329–337.
39. Wilcox, K. W., Kohn, A., Sklyanskaya, E. & Roizman, B. (1980) *J. Virol.* **33,** 167–182.
40. Morse, L. S., Pereira, L., Roizman, B. & Schaffer, P. A. (1978) *J. Virol.* **26,** 389–410.
41. Preston, C. M. (1979) *J. Virol.* **29,** 275–284.
42. Dixon, R. A. F. & Schaffer, P. A. (1980) *J. Virol.* **36,** 189–203.
43. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50,** 349–383.
44. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217,** 316–324.
45. McKnight, S. L. (1982) *Cell* **31,** 355–365.