Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 71–75, January 1987
Biochemistry

Host cell proteins bind to the cis-acting site required for virion-mediated induction of herpes simplex virus 1 α genes

(α-trans-induction factor/gel assay/DNase I/DNA–protein complexes)

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ABSTRACT **The herpes simplex virus 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. In productively infected cells, the α genes are expressed first, and a virion protein, the α-trans-inducing factor (α-TIF), acts in trans to enhance their expression. Induction of the α genes by α-TIF requires the presence of a trans-induction cis-acting site (α-TIC), and one to three homologs of the α-TIC sequence are contained in the regulatory domains of all α genes. We report that small DNA fragments from regulatory domains of *α0*, *α4*, and *α27* genes containing α-TIC homologs formed complexes with host but not viral proteins. DNase protection studies indicated that the major host protein complex α–H1 detected in DNA gel retardation assays bound asymmetrically across the α-TIC site. All DNA fragments containing α-TIC homologs, but not those lacking the homolog, competed for the binding of this complex. The location of the binding site of the other host proteins is not yet known. Simian virus 40 DNA fragments containing a homolog of the α-TIC sequence also competed with herpes simplex virus DNA fragments carrying authentic α-TIC homologs for the α–H1 protein complex.**

The genes encoded in the herpes simplex virus type 1 (HSV-1) genome form several groups whose synthesis is coordinately regulated and sequentially ordered in a cascade fashion (ref. 1; reviewed in ref. 2). The five α genes, *α0*, *α4*, *α22*, *α27*, and *α47*, are the first to be transcribed, and at least one functional α protein, α4, is required for the expression of β and γ genes transcribed later in infection (2–5). Recently, we and others have shown that the α4 protein binds to specific sites in the promoter–regulatory domains of α, β, and γ genes (6–8, 38). The sites of binding of the α4 protein in the domains of α genes are consistent with studies showing that the expression of α genes is regulated, at least in part, by the products of the α4 gene and that this *trans*-acting protein regulates transcription both positively and negatively.

A remarkable property of HSV-1 gene regulation is that a structural protein located in the tegument (i.e., between the capsid and the envelope of the virion) induces the expression of α genes in trans (9, 10). The gene specifying the α-trans-inducing factor (α-TIF) has been mapped and sequenced (11–14). Studies on chimeric genes consisting of the promoter–regulatory domains of α genes fused to an indicator gene have shown that α genes contain a sequence that acts as a cis site for the induction of α genes by α-TIF (14–20). Homologs of this α-trans-induction cis site (α-TIC) are present in at least one (*α27* gene) to three copies (*α0* gene) in the regulatory domains of α genes (15).

In the course of studies on the binding of α4 protein to HSV-1 DNA, we noted that DNA fragments containing the α-TIC sequence bind specifically cellular rather than virally encoded proteins (6, 7). In this paper we report that at least one cellular protein binds specifically to the α-TIC site. These data suggest that, in contrast to the α4 protein, the trans-acting function of α-TIF is mediated through its effect on one or more host proteins.

MATERIALS AND METHODS

Cells, Virus, and Protein Extracts. HeLa cells, grown to confluency in 850-cm^2 roller bottles, were mock-infected or infected with 5 plaque-forming units of HSV-1 strain F [HSV-1(F)] (21) and maintained for 2 hr or 12 hr. Nuclear extracts (8–9 mg of protein per ml) were prepared as described (22).

Preparation of DNA Probes and Competitor DNAs. The cloning of the DNA fragments shown in Fig. 1*B* has been described (6, 7, 19). For DNA probes, the appropriate plasmid DNA was digested with *Eco*RI and *Hin*dIII, dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim), purified from polyacrylamide gels, and 5′-end labeled with [γ-^{32}P]ATP (>7000 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) by phage T4 polynucleotide kinase (United States Biochemical, Cleveland, OH) to an activity of 25,000 to 50,000 cpm/ng of DNA fragment (23, 24). All DNA fragments used in the competition assays were purified from restriction enzyme digests of CsCl gradient-purified plasmid DNAs separated by polyacrylamide gel electrophoresis as described. The concentrations of all competitor DNA fragments were determined by comparison with known standards in agarose gels.

RESULTS

One or More Host Proteins Bind to DNA Fragments Containing the α-TIC of α Genes *0*, *4*, and *27*. Fig. 1*B* shows the genomic locations of the four DNA fragments tested for their ability to bind host cell and viral-encoded proteins in the presence of excess competitor nucleic acids using the gel retardation assay originally described by Fried and Crothers (28) and Garner and Revzin (29). 150α4R and 70α4R represent the promoter proximal 150-base-pair (bp) and promoter distal 70-bp portions of the *α4* gene regulatory domain, respectively. 48α27R and 29α0R designate a 48-bp and a 29-bp sequence cloned from the regulatory domains of the *α27* and *α0* genes, respectively. Each of these cloned DNA fragments contains a homolog of the α-TIC sequence required for induction of α indicator genes by α-TIF (15). These fragments were labeled with [γ-^{32}P]ATP and incubated, in the presence of excess competitor nucleic acids, with protein

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Abbreviations: HSV-1, herpes simplex virus 1; HSV-1(F), HSV-1 strain F; bp, base pair(s); α-TIF, α-trans-inducing factor; α-TIC, α-trans-induction cis-acting site; α-H$_x$, host protein(s)$_x$ binding to α-TIC; SV40, simian virus 40; CAT, chloramphenicol acetyltransferase.

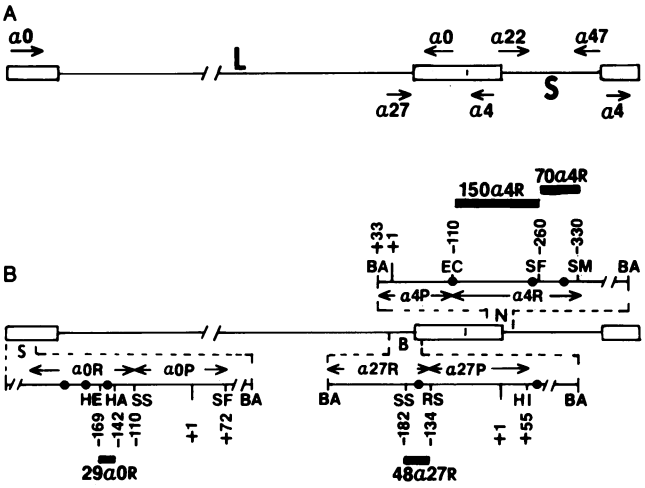

FIG. 1. (*A*) Sequence arrangement of the HSV-1 genome and location of the α genes. The genome is shown in the prototype arrangement (25). The boxed regions represent the inverted repeat sequences *ba* and *b'a'* flanking the L (long) component and *a'c'* and *ac* flanking the S (short) component. Arrows indicate the location and orientation of the genes encoding the α proteins 0, 4, 27, 22, and 47 (26, 27). (*B*) Sequence arrangement of the promoter–regulatory domains of α genes *0*, *27*, and *4*. The expanded scales show the restriction patterns and the locations of the regulatory (R) and promoter (P) domains of *α0*, *α27*, and *α4* located within the viral *Bam*HI DNA fragments S, B, and N, respectively. The filled circles indicate the location of the α-TIC homologs (15). Bold lines delineate the DNA fragments derived from the α-gene regulatory domains tested in these studies. All numbers refer to the site of transcription initiation. HE, *Hae* III; HA, *Hae* II; SS, *Sac* II; SF, *Sfa*NI; BA, *Bam*HI; RS, *Rsa* I; HI, *Hin*fI; EC, *Eco*RI; SM, *Sma* I.

extracts of cells mock-infected, infected with HSV-1(F) for 2 hr, or infected with HSV-1(F) for 12 hr. The resulting complexes were electrophoretically separated in a nondenaturing gel and visualized by autoradiography (Fig. 2).

For each fragment and extract tested, the amounts of protein extract and competitor nucleic acids were 1 μg and 1.7 μg or 5.0 μg and 5.2 μg. The following results were obtained. (*i*) The pattern of DNA–mock-infected protein complexes did not differ from that of complexes formed with proteins from 2- or 12-hr HSV-1-infected cells. (*ii*) Each of the DNA fragments formed a major comigrating DNA–protein complex (designated α–H1) at the higher protein/competitor nucleic acid ratio with all of the extracts tested. A comigrating DNA–protein complex was also formed in the lower protein/competitor ratio reaction with the 70α4R DNA probe. Several minor 48α27R DNA–protein complexes were also evident (designated α–H2 and α–H3).

Specificity of the Protein–DNA Binding in the Major α–H1 Protein–DNA Complexes. Fig. 3 shows the results of a series of competition studies designed to determine the specificity of the protein–DNA sequence interactions in the major, comigrating DNA–protein complexes (designated α–H1 in Fig. 2). In these studies, labeled DNA fragments 48α27R (Fig. 3*A*) and 29α0R (Fig. 3*B*) were incubated with mock-infected cell extract in the presence of increasing quantities of various unlabeled competitor DNA fragments. The unlabeled homologous 48α27R competitor DNA, but not the control fragments (PUC and CAT), efficiently competed with the labeled 48α27R probe for the DNA-binding proteins in the α–H1 complex (Fig. 3*A*). The 29α0R, the 70α4R, and the 150α4R fragments also competed for the proteins in this complex. It is of interest to note that the SV40 enhancer–early promoter region also competed efficiently, in contrast to the α-gene promoter and the metallothionein-gene promoter DNA fragments. Therefore, these results suggest that the proteins involved in the α–H1 complex are sequence-specific binding proteins, recognizing a motif common to the four α DNA fragments and also common to the SV40 early promoter

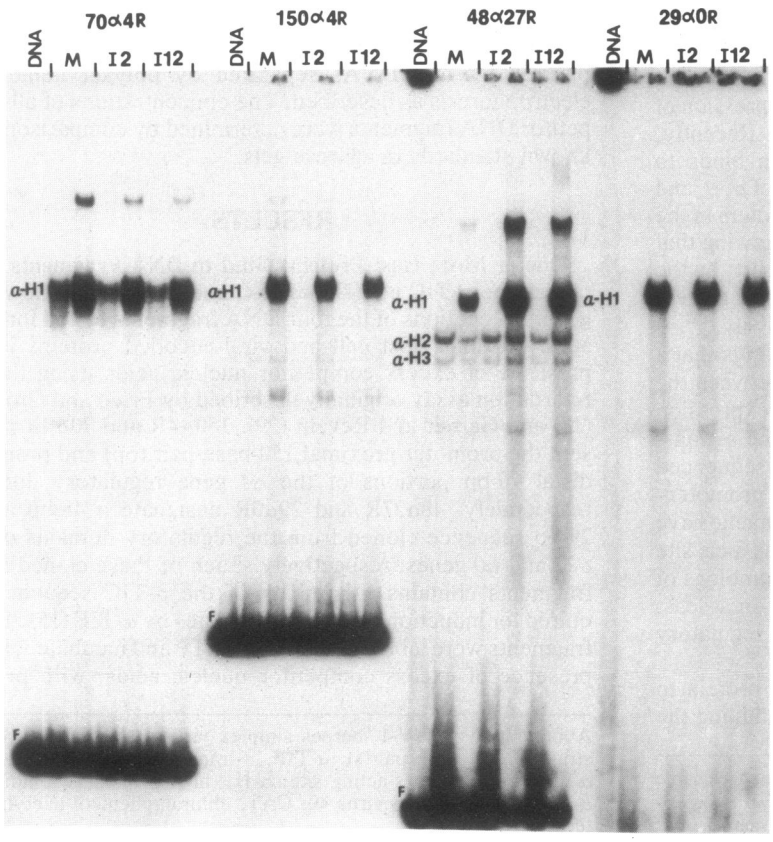

FIG. 2. Autoradiographic images of labeled DNA–protein complexes subjected to electrophoresis in nondenaturing gels. The DNA fragments from α-gene regulatory domains were complexed with proteins from mock- and HSV-1-infected cells as described (6, 7). Specifically, labeled DNA probes (0.5 ng) were incubated with protein extract in the presence of poly(dI)·poly(dC) (Pharmacia P-L Biochemicals) in 20 mM Tris·HCl, pH 7.6/50 mM KCl/1 mM EDTA/0.05% Nonidet P-40/5% (vol/vol) glycerol/5 mM 2-mercaptoethanol/50 μg of bovine serum albumin (Sigma) per ml in a reaction volume of 15 μl for 45 min at 25°C. The reaction mixtures were electrophoretically separated in a 4% polyacrylamide gel, dried, and exposed to Kodak XS film for 12–16 hr. Lanes marked DNA contain the DNA probes only. The protein extract tested is indicated at the top of each pair of reactions as follows: M, extract from mock-infected cells; I2, extract from cells infected with HSV-1(F) for 2 hr; and I12, extract from cells infected with HSV-1(F) for 12 hr. The first lane of each pair represents reactions with 1 μg of protein extract and 1.7 μg of poly(dI)·poly(dC), whereas the second lane represents reactions with 5 μg of protein extract and 5.2 μg of poly(dI)·poly(dC). The protein–DNA complexes are designated α–H1, α–H2, and α–H3, whereas the position of the free DNA probe is indicated by F. The unbound DNA of 29α0R fragment migrates slightly faster than that of 48α27R and is not represented on this gel.

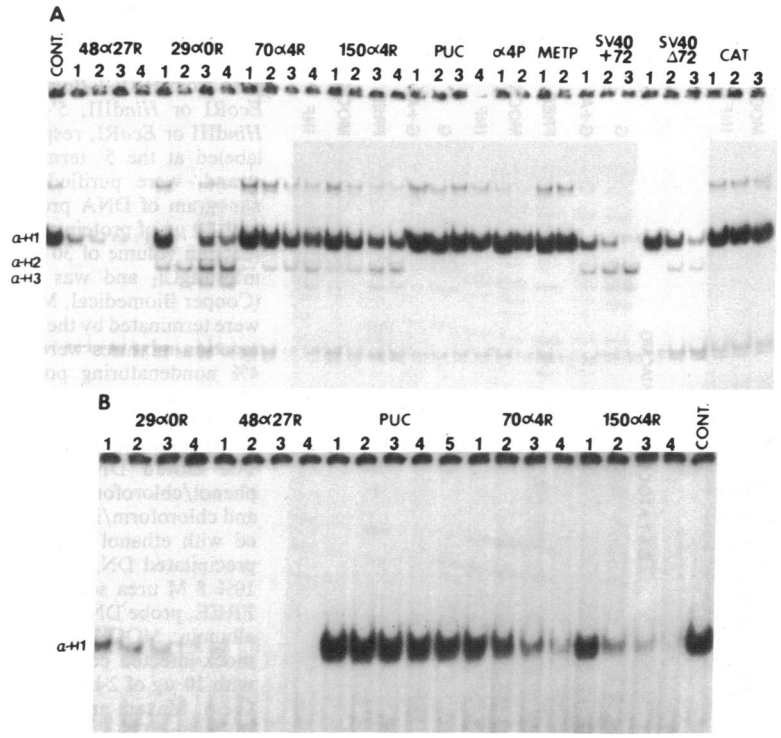

FIG. 3. Autoradiographic images of labeled DNA–protein complexes formed in the presence of various competitor DNAs and electrophoretically separated in nondenaturing gels. DNA–protein binding assays were done as described in the legend to Fig. 2 with 5 μg of mock-infected cell protein and 5.2 μg of poly(dI)·poly(dC) per reaction. In addition, increasing concentrations of the unlabeled DNA fragment indicated at the top of the lanes were added to the reaction mixture prior to the addition of the protein extract as follows: lanes 1, 0.15 pm; lanes 2, 0.38 pm; lanes 3, 0.78 pm; lanes 4, 1.17 pm; and lanes 5, 1.4 pm (representing approximately 15-, 38-, 78-, 117-, and 140-fold molar excess over the probe DNA concentration). The DNA fragments derived from α-gene regulatory domains and used as competitor DNAs are shown in Fig. 1. PUC, the 175-bp *Pvu* II–*Hin*dIII fragment of pUC9 (30); α4P, the promoter domain of the α4 gene extending from +33 to −110 relative to the transcription initiation site (16); METP, the 216-bp *Sac* I-*Bgl* II fragment derived from pMK (gift of R. Palmiter) (31) and containing the mouse metallothionein gene promoter domain; SV40 +72, the 340-bp *Pvu* II–*Hin*dIII fragment of pSV2cat (gift of G. Khoury), which contains the SV40 early promoter and both enhancer repeats (32); SV40 Δ72, the 200-bp *Bgl* II–*Hin*dIII fragment of pA10cat (gift of G. Khoury), which contains the SV40 early promoter and ≈0.25 of the origin–proximal enhancer element (32); CAT, the 160-bp *Pvu* II–*Hin*dIII fragment of pSV2cat and containing a portion of the chloramphenicol acetyltransferase (CAT) gene coding sequences; CONT., control reaction in the absence of competitor DNA fragments. Protein–DNA complexes are labeled α–H1, α–H2, and α–H3 as in Fig. 2. (*A*) Competition for proteins involved in the formation of the 48α27R DNA probe–protein complexes. (*B*) Competition for proteins involved in the formation of the 29α0R DNA probe–protein complexes.

region. A potentially significant observation was the enhancement of the formation of the 48α27R α–H2 and α–H3 complexes as the formation of the 48α27R α–H1 complex was reduced by competing DNA framents.

In the reciprocal experiment, shown in Fig. 3*B*, the four unlabeled fragments from the α-gene regulatory domains, but not the control fragments, competed with the labeled 29α0R fragment for the proteins in the major α–H1 complex. As expected from the first series of competition analyses, the 48α27R fragment competed most efficiently for the proteins involved in the 29α0R major complex (15× molar excess). The homologous 29α0R, 70α4R, and 150α4R fragments also competed significantly relative to the control PUC competitor DNA. The competition series emphasizes the specificity of the binding of the proteins in the α–H1 complex to DNA sequences common to the four α-gene fragments. In contrast to the results obtained in Fig. 3*A* with the 48α27R labeled probe, no secondary protein–DNA complexes were formed by competition for the 29α0R major complex proteins, supporting the conclusion that the proteins involved in 48α27 α–H2 and α–H3 formation are unrelated to those involved in α0-fragment α–H1 formation.

DNase I Cleavage-Inhibition Patterns of the Major α-TIC Protein–DNA Complex. To determine the binding site of the protein(s) involved in the formation of the α-H1 protein–DNA complex, the 48α27R fragment was labeled with γ-^{32}P at the 5′ end of the coding and noncoding strands, incubated with mock-infected or 2-hr HSV-1(F)-infected extract, and partially digested with DNase I (33). The α–H1 complex and the unbound DNA were electrophoretically separated on a nondenaturing gel, eluted from the gel, and electrophoretically separated on a standard denaturing gel as described (24, 34). The autoradiographic images of the coding- and noncoding-strand domains protected from DNase I digestion are shown in Fig. 4. The protected domain in both cases was unaltered by increased concentrations of DNase I (Fig. 4, lanes B). Comparisons of the protected domains of the DNA fragment treated with extracts from mock-infected cells with those obtained with extracts of 2-hr HSV-1(F)-infected cells showed no significant differences, in accord with the conclusion that the proteins involved in the α–H1 complex are of host origin.

Fig. 5 shows the location of the nucleotide sequences of the 48α27R fragment protected from DNase I digestion by the protein(s) in the α–H1 complex. The protected domain extends for 20 nucleotides on each strand and appears to be asymmetrically positioned on the coding and noncoding strands. Significantly, the protected regions coincide with the α-TIC, present in the regulatory domains of all α genes, and required for the *in vivo* regulation of an α gene by α-TIF. Preliminary data indicates that the α-TIC homolog in 29α0R is similarly protected by the proteins of the 29α0R α–H1 complex. As could be predicted, the SV40 DNA sequences sharing homology with α-TIC homologs successfully com-

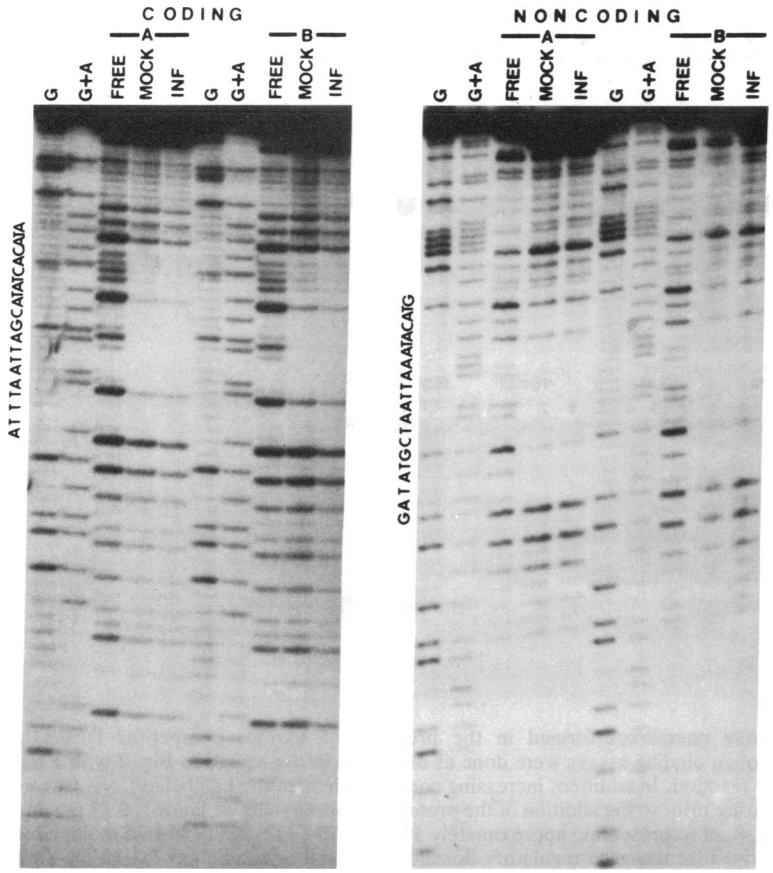

FIG. 4. DNase protection analyses of α–H1 factor binding to 48α27R DNA. pRB606, containing the 48-bp *Sac* I–*Rsa* I fragment of the regulatory domain of the α27 gene from HSV-1 *Bam*HI B fragment, was digested with *Eco*RI or *Hin*dIII, 5′-end-labeled, and redigested with *Hin*dIII or *Eco*RI, respectively. The resulting fragments, labeled at the 5′ terminus of the noncoding or coding strand, were purified from polyacrylamide gels. One nanogram of DNA probe (≈50,000 cpm) was incubated with 10 μg of protein and 10.4 μg of poly(dI)·poly(dC) per reaction volume of 30 μl in binding buffer containing 2.5 mM $MgCl_2$ and was partially digested with DNase I (Cooper Biomedical, Malvern, PA). The DNase reactions were terminated by the addition of EDTA to 5 mM, and the reaction mixtures were subjected to electrophoresis in a 4% nondenaturing polyacrylamide gel. After autoradiography to visualize the DNA–protein complexes and the unbound DNA, the DNA was eluted from each position in 0.5 M ammonium acetate/1 mM EDTA/0.1% $NaDodSO_4$. The eluted DNA was extracted sequentially with phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol), and chloroform/isoamyl alcohol, 24:1, and was precipitated with ethanol in the presence of carrier tRNA. The precipitated DNA was resuspended and separated on a 10% 8 M urea sequencing gel as described (24). Lanes: FREE, probe DNA incubated with 10 μg of bovine serum albumin; MOCK, probe DNA incubated with 10 μg of mock-infected cell protein; INF, probe DNA incubated with 10 μg of 2-hr HSV-1(F)-infected cell protein; G and G+A, Maxam and Gilbert specific cleavage reactions of the probe DNA (24). Reaction mixtures A and B were incubated with 2.5 or 5.0 μg of DNase I per ml, respectively, for 2.5 min at 25°C. (*Left*) CODING refers to 48α27R probe DNA labeled at the 5′ terminus of the coding strand. (*Right*) NONCODING refers to 48α27R probe DNA labeled at the 5′ terminus of the noncoding strand. The nucleotide sequences of 48α27R DNA protected from DNase I cleavage are indicated to the left of lanes G.

peted with DNA fragments containing authentic α-TIC homologs for the α–H1 protein(s). In this regard, the fragment that contains two intact α-TIC sequence homologs (SV40 +72) competed more efficiently than the fragment that contains one truncated homolog (SV40 Δ72) for the α–H1 proteins (Fig. 3*A*).

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48αα27R DNase protection
noncoding                          >>>>>>>>>>>>>>>>>>>>
          CGGAAGCGGAACGGTGTATGTGATATGCTAATTAAATACATGCCACGT
          GCCTTCGCCTTGCCACATACACTATACGATTAATTTATGTACGGTGCA
coding                        <<<<<<<<<<<<<<<<<<<<

                                    C       G     T
αα-TIC homolog consensus            G-ATGNTAATGA-ATTC-TTGNGGG
                                    T             A     C

αα-TIC sites in test fragments
     48αα27R                        ATATGCTAATTAAATACATGCCACG
     29αα0R                         GCATGCTAATGATATTCTTTGGGGG
     70αα4R                         GCATGCTAACGAGGAACGGGCAGGG
    150αα4R and                        CGGTAATGAGATGCCATGCGGG
                                    CGTGCATAATGGAATT

αα-TIC homologs in SV40 DNA
    Δ72 competing fragment          GCTGACTAATtGAGAT
    +72 competing fragment          GCTGACTAATtGAGATGCATGCTTTG
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FIG. 5. The nucleotide sequence of the 48α27R DNA fragment and domains of the noncoding (>) and coding (<) strands protected from DNase I digestion by α–H1 host protein complex. Line 6 shows the α-TIC noncoding strand consensus sequence as reported (15). Below the consensus sequence are the nucleotide sequences of the α-TIC (noncoding strand) homologs present in the DNA fragments, derived from α-gene regulatory domains and tested in this study, and the homologous sequences of the SV40 DNA fragments containing enhancer domains and designated +72 or Δ72. The SV40 DNA fragments are described in the legend to Fig. 3. Only one of the two repeated homologs in the +72 DNA fragment is shown. The 150α4R fragment contains the intact promoter distal and a portion of the promoter proximal α-TIC homologs. All sequences are written 5′ to 3′.

DISCUSSION

The Binding of the α–H1 Proteins to the cis-Site Required for the Induction of α-Gene Expression. The promoter–regulatory domains of α genes can be subdivided into a promoter domain, which imparts upon the indicator gene the capacity to be expressed upon transfection into eukaryotic cells, and a regulatory domain, which imparts high-basal-level expression and the capacity to be induced and regulated as an α gene (16, 19). The induction of α-gene expression is mediated by an HSV-1 virion component, α-TIF, in trans and requires a small DNA element containing a homolog common to all α-gene regulatory domains (α-TIC) (14, 15, 19). Attempts to demonstrate that α-TIF binds to the α-TIC homologs have not been successful. This report demonstrates that the DNA fragments containing the α-TIC homolog bind host proteins and that these complexes form several discrete bands in DNA gel retardation assays. In the case of the major protein–48α27 DNA fragment complex, the DNase protection studies indicate that the protein binding site overlaps the α-TIC homolog. Moreover, the other fragments containing the α-TIC homolog successfully competed with the 48α27R fragment for the proteins in this complex, indicating that these fragments also contain the binding site for these proteins. For convenience, we shall refer to the proteins in the major protein–DNA complex as the α gene–host binding protein 1 complex or α–H1.

Titrations of cell extracts suggest that the binding of α–H1 proteins to the α-TIC homologs is strongly concentration dependent. It is conceivable, therefore, that the band formed by α–H1 may involve multiple proteins rather than monomeric or oligomeric proteins and that the stability of the complex depends upon the presence of all proteins.

The Binding of Secondary (α–H2, α–H3) Proteins to DNA Fragments Containing the α-TIC Site. Low concentrations of protein extracts with the 48α27R fragment yielded several minor bands migrating faster than the α–H1 DNA complex, in contrast to the reactions with the 29α0R fragment. The evidence that the protein in these complexes (α–H2 and α–H3 in Figs. 2 and 3) may not be related to α–H1 rests on competition studies. Pending the DNase I inhibition pattern of the binding site(s) of α–H2 and α–H3 in the 48α27R fragment, their concordance with the α-TIC homolog and their specificity remain in question. It should be noted that *in vivo* studies with chimeric α genes produced by fusion of the 48α27R or the 29α0R fragments to the promoter domains of the α4 or the β thymidine kinase gene have suggested that, although the α-TIC homolog contained within both α donor fragments is necessary for α-gene regulation, additional sequence elements contained only within the larger 48α27R fragment were both necessary and sufficient to regulate the heterologous β-class promoter as an α gene (19). Therefore, it would be consistent that the 48α27R fragment contain additional cis-acting sites that would be lacking in the 29α0R fragment.

The Nature of the Host Proteins Binding to the α-TIC Homolog. The nature of the proteins with affinity for the α-TIC homolog is not known. Consistent with the results reported here, we noted earlier (20) that homologs of the consensus sequence for the α-TIC site are present in promoter–regulatory domains of several inducible eukaryotic genes. *In vivo*, the α-TIF protein will induce the expression of other viral α or immediate early genes such as the 95-kDa protein of the human cytomegalovirus in cells permissive for cytomegalovirus growth (35). These observations suggest that the proteins binding to the α-TIC homolog may represent a subset of transcriptional factors involved in the expression of inducible genes. HSV-1 has a wide experimental host range. It would follow that, if induction of α genes is required for viral multiplication, all of the proteins interacting with the DNA fragments containing the α-TIC site and capable of conferring α-gene regulation upon promoter elements must be well conserved among vertebrates.

How Does α-TIF Induce the Expression of the α Genes? In contrast to the α4 protein, the major HSV regulatory protein required for the induction of HSV-1 β and γ genes, the α-TIF does not appear to bind to viral DNA. The absence of demonstrable binding is not *per se* conclusive, but the observation that the cis-acting site required for induction of α genes by α-TIF specifically binds host proteins suggests that α-TIF may, in fact, effect transcription of α genes by modifying host transcriptional factors rather than by binding directly to viral DNA. In this respect, α-TIF may resemble the E1A protein of adenoviruses (36) rather than the α4 protein of HSV-1 (6–8, 38).

The size and nucleotide sequence of α-gene promoter–regulatory domains suggest that they contain cis-acting sites for a variety of transcriptional factors but that the distribution of these factors among the α genes is not uniform. No fewer than four SP1 transcriptional factor binding sites have been mapped within the α4 promoter–regulatory domain (37), but its consensus sequence is not present in the domains of all of the α genes. It is conceivable that each α gene has a nonuniform set of cis-acting sites for a variety of transcriptional factors which, in concert, determine the level and other features of the expression of each gene, but that the initiation of high-level transcription would be determined by activation of a subset of these transcriptional factors. In contrast to the α4 protein, which appears to be able to regulate gene expression both positively and negatively, the operational effect of α-TIF would be solely positive, but its mode of action is less clear at this time. The apparent multiplicity of host proteins binding to small DNA fragments containing the α-TIC homolog does not allow us to determine at this time if α-TIF inactivates the major binding proteins (α–H1), enabling the minor ones (e.g., α–H2 and α–H3) to enhance transcription, or activates the existing binding protein, thereby changing the rate of transcription.

We thank G. Khoury for the gift of plasmids pSV2cat and pA10cat and R. Palmiter for the gift of plasmid pMK. These studies were aided by grants from the National Cancer Institute (CA08494 and CA19264) and the American Cancer Society (MV2T).

1. Honess, R. W. & Roizman, B. (1974) *J. Virol.* **14,** 8–19.
2. Roizman, B. & Batterson, W. (1985) in *Virology*, ed. Fields, B. (Raven, New York), pp. 497–526.
3. Watson, R. J. & Clements, J. B. (1980) *Nature (London)* **285,** 329–330.
4. Preston, C. M. (1979) *J. Virol.* **29,** 275–284.
5. Dixon, R. A. F. & Schaffer, P. A. (1980) *J. Virol.* **36,** 189–203.
6. Kristie, T. M. & Roizman, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3218–3222.
7. Kristie, T. M. & Roizman, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4700–4704.
8. Beard, P., Faber, S., Wilcox, K. W. & Pizer, L. I. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4016–4020.
9. Post, L. E., Mackem, S. & Roizman, B. (1981) *Cell* **24,** 555–565.
10. Batterson, W. & Roizman, B. (1983) *J. Virol.* **46,** 371–377.
11. Campbell, M. E. M., Palfreyman, J. W. & Preston, C. M. (1984) *J. Mol. Biol.* **180,** 1–19.
12. Pellett, P., McKnight, J. L. C., Jenkins, F. & Roizman, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5870–5874.
13. Dalrymple, M. A., McGeoch, D. J., Davison, A. J. & Preston, C. M. (1985) *Nucleic Acids Res.* **13,** 7865–7879.
14. McKnight, J. L. C., Kristie, T. M., Silver, S., Pellett, P. E., Mavromara-Nazos, P., Campadelli-Fiume, G., Arsenakis, M. & Roizman, B. (1986) in *Cancer Cell: Control of Gene Expression and Replication*, eds. Botchan, M., Grodzicker, T. & Sharp, P. (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY), Vol. 4, pp. 163–173.
15. Mackem, S. & Roizman, B. (1982) *J. Virol.* **44,** 939–949.
16. Mackem, S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4917–4921.
17. Mackem, S. & Roizman, B. (1982) *J. Virol.* **43,** 1015–1023.
18. Post, L. E., Mackem, S. & Roizman, B. (1981) *Cell* **24,** 555–565.
19. Kristie, T. M. & Roizman, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4065–4069.
20. 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.
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. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
24. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
25. Roizman, B. (1979) *Annu. Rev. Genet.* **13,** 25–57.
26. Mackem, S. & Roizman, B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7122–7126.
27. Rixon, F. & Clements, J. (1982) *Nucleic Acids Res.* **10,** 2241–2256.
28. Fried, M. & Crothers, D. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
29. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
30. Vieria, J. & Messing, J. (1982) *Gene* **19,** 259–268.
31. Brinster, R. L., Chen, H. Y., Warren, R., Sarthy, A. & Palmiter, R. D. (1982) *Nature (London)* **296,** 39–42.
32. Laimins, A. L., Khoury, G., Gorman, C., Howard, B. & Gruss, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6453–6457.
33. Galas, D. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3127–3170.
34. Singh, H., Sen, R., Baltimore, D. & Sharp, P. A. (1986) *Nature (London)* **319,** 154–158.
35. Spaete, R. R. & Mocarski, E. S. (1985) *J. Virol.* **56,** 135–143.
36. Kovesdi, I., Reichel, R. & Nevins, J. R. (1986) *Science* **231,** 719–722.
37. Jones, K. A. & Tjian, R. (1985) *Nature (London)* **317,** 179–185.
38. Faber, S. W. & Wilcox, K. W. (1986) *Nucleic Acids Res.* **14,** 6067–6083.