Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7061–7065, October 1987
Biochemistry

Binding of the virion protein mediating α gene induction in herpes simplex virus 1-infected cells to its cis site requires cellular proteins

(trans-acting factor/protein–DNA complexes/band-shift assays/transcriptional regulation/*in vitro* transcription–translation)

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ABSTRACT **In herpes simplex virus 1-infected cells, the transcription of α genes, the first set of genes to be expressed, is induced by a virion component, the α-trans-induction factor, and requires a cis site. Homologs of the cis site are present in the promoter-regulatory domains of all α genes and bind two cellular proteins designated as αH1 and αH2–αH3. We report that α-trans-induction factor, synthesized *in vitro* or present in nuclear extracts of infected cells, forms complexes with viral DNA fragments containing its cis-acting site only in the presence of cellular proteins and only under conditions that also enable the binding of the αH1 protein to the DNA. The induction of α genes by α-trans-induction factor appears, therefore, to be mediated by the interaction of the viral protein with cellular proteins at its cis-acting site.**

Herpes simplex virus 1 (HSV-1) gene expression is tightly regulated in a cascade fashion (1). A distinctive feature of HSV-1 gene regulation is that a virion protein induces the α genes, the first set of genes transcribed after infection (2, 3). The γ gene encoding this α-trans-induction factor (α-TIF) has been mapped, sequenced, and cloned (4–7). These studies identified α-TIF as the infected cell protein 25 (ICP25; apparent M_r, 64,000), contained in 500–1000 copies per virion between the envelope and the capsid, and confirmed earlier studies that determined that α-TIF-mediated induction requires the presence of an A+T-rich sequence found in the promoter-regulatory domains of all α genes, termed the α-trans-induction cis-acting (α-TIC) site (reviewed in ref. 8; see also refs. 6, 9–13). The uniqueness of this model system of gene regulation led us to determine whether α-TIF binds to its cis sites on viral DNA.

Attempts to demonstrate the binding of α-TIF to the α-TIC site have been unsuccessful. However, these studies have revealed that the nuclear extracts of cultured cells contain two major proteins that specifically bind to the domain of the α-TIC site (14). The first, designated αH1, with a maximum apparent molecular weight of 110,000, protects the domain of the α-TIC site from DNase I digestion, and its protein–purine contacts, revealed by methylation interference studies, consist primarily of adenines clustered at the 5′ terminus of the α-TIC site (ref. 14; T.M.K. and B.R., unpublished results). The second protein αH2-αH3 is chromatographically distinct, has a maximum apparent molecular weight of 64,000, and its binding site, rich in guanines, is on the 3′ side of and partially overlaps the αH1 binding site (ref. 14; T.M.K. and B.R., unpublished results). αH1 and αH2-αH3 can bind concurrently to DNA fragments containing the α-TIC site (14). In this paper we report that α-TIF binds to a complex containing the α-TIC site and fractions containing the αH1 but not the αH2-αH3 proteins. The presence of α-TIF in DNA–protein complexes rests on the demonstration that the complex contained the α-TIF protein, radioactively labeled during *in vitro* synthesis, and that antibody to a synthetic peptide predicted from the nucleotide sequence of the α-TIF gene retarded the migration of the α-TIF DNA–protein complex in band-shift assays.

MATERIALS AND METHODS

Virus and Cells. The properties of HSV-1(F), the prototype HSV-1 strain used in this laboratory, have been described elsewhere (15). Both HeLa and Vero cells were maintained in Dulbecco's modified Eagle's medium in 5% (vol/vol) fetal calf serum in an atmosphere of 95% air/5% CO_2.

Preparation of Cellular Extracts. Confluent monolayers of HeLa cells were grown in 850-cm^2 roller bottles, mock-infected or infected with 5 plaque-forming units/ml of HSV-1(F), and harvested 12 hr after infection. Nuclear fractions were prepared as described (16). The αH1 and αH2-αH3 proteins were chromatographically fractionated from mock-infected HeLa cell nuclear extracts as described (T.M.K. and B.R., unpublished results).

Preparation of DNA Probes. The cloning and preparation of the DNA probes used are described elsewhere (14, 17).

Gel Retardation Assays. The gel retardation assays follow the procedures originally described by Fried and Crothers (18) and by Garner and Revzin (19), and have been described elsewhere (14).

RESULTS

***In Vitro* Synthesis of α-TIF.** To synthesize the HSV-1 α-TIF *in vitro*, a 1728-base-pair (bp) DNA sequence, derived from the HSV-1 *Bam*HI F DNA fragment and consisting of the intact structural domain of α-TIF and 64 bp of 5′-leader sequence, was inserted into the pGEM-1 transcriptional vector under the control of the bacteriophage SP6 promoter as pRB3717 (Fig. 1). α-TIF mRNA was transcribed *in vitro* from pRB3717 DNA in the presence of the mRNA cap structure analog 7-methylguanosine(5′)triphospho(5′)guanosine [m^7G(5′)ppp(5′)G] to increase translational efficiency (26). The single major mRNA species obtained by this procedure, corresponding in size (≈1.65 kilobases) to that predicted from the nucleotide sequence for nonpolyadenylylated α-TIF mRNA (6), was translated *in vitro* in [^{35}S]methionine (Fig. 2, lanes 2–4). The single, major ^{35}S-labeled protein synthesized *in vitro* had an apparent molecular weight of 64,000 and comigrated with HSV-1 ICP25 (α-TIF) (lane 1). The translation reaction, in the absence of α-TIF mRNA, did not yield any ^{35}S-labeled proteins (lane 5).

Characterization of a Rabbit Antibody Directed Against a Synthetic Peptide Representing a Domain from the α-TIF Protein

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Abbreviations: HSV-1, herpes simplex virus 1; HSV-1(F), HSV-1 strain F; α-TIF, α-trans-induction factor; α-TIC, α-trans-induction cis-acting site; ICP, infected cell protein.

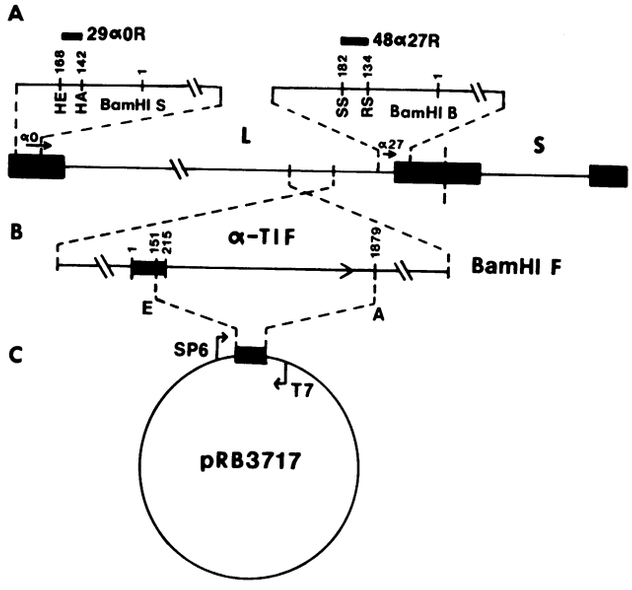

FIG. 1. (*A*) Schematic representation and relevant maps of the HSV-1 genome in the prototype orientation. Solid rectangles, inverted repeat sequences that flank the L (long) and the S (short) components of the viral genome (20). The expanded regions show the transcriptional orientation, relevant restriction sites, and domains of the genes encoding α0, α27, and α-TIF on *Bam*HI S, B, and F DNA fragments, respectively (6, 21). The numbers indicate the nucleotide positions of the restriction endonuclease cleavage sites relative to the transcriptional initiation sites located downstream at nucleotide 1. HA, *Hae* II; HE, *Hae* III; RS, *Rsa* I; SS, *Sst* II. (*B*) Expanded map of the *Bam*HI F restriction fragment containing the α-TIF gene. Numbers refer to the nucleotide positions of the indicated restriction sites, and the initiating ATG codon (nucleotide 215) on the 3′ side of the transcriptional initiation site at nucleotide 1 (6). E, *Eco*RV; A, *Asu* II. (*C*) Structure of pRB3717 for *in vitro* transcription. pRB3722, the plasmid intermediate of pRB3717, was constructed by the insertion of the 1728-bp *Eco*RV–*Asu* II fragment isolated from pRB3458 (described in ref. 6) into the *Sma* I site of pUC19, after treatment with T4 polymerase (New England Biolabs) (22). The 1728-bp fragment was excised from pRB3722 with *Eco*RI and *Hin*dIII and cloned as pRB3717 into the *Eco*RI–*Hin*dIII site of the pGEM-1 transcriptional vector (Promega Biotec, Madison, WI) under the control of the bacteriophage SP6 promoter. *In vitro* transcription was done on 0.3 μg of pRB3717 DNA digested with *Hin*dIII according to the protocol and reagents supplied by Promega Biotec (23), for the synthesis of large amounts of unlabeled RNA. The RNA cap structure analog 7-methylguanosine(5′)triphospho(5′)guanosine [m^7G(5′)ppp(5′)G] (12.5 mM, New England Biolabs) was included in the transcription reaction mixture to synthesize capped mRNAs (24). The mRNA was extracted with phenol/chloroform, 1:1 (vol/vol), with chloroform and stored in 2.5 volumes of 95% (vol/vol) ethanol at −20°C. The concentration and size of the α-TIF mRNA were relative to rRNA standards (25).

as Predicted from Its Sequence. The experiments in Fig. 2*B* demonstrate that rabbit antibody, directed against a 12-amino acid sequence contained within α-TIF, reacts with authentic ICP25 (α-TIF). The reaction of the rabbit anti-peptide antibody with mock-infected cell lysates, electrophoretically separated in denaturing polyacrylamide gels and electrically transferred to nitrocellulose (Fig. 2, lanes 7 and 8), are shown in Fig. 2, lanes 9 and 10. The serum reacted with a protein band comigrating with ICP25 (α-TIF) in lysates of infected cells (lane 10) but not in lysates of uninfected cells (lane 9).

***In Vitro* Translated α-TIF Forms a Complex with the HSV-1 48α27R DNA Fragment.** The experiments described in this section show that α-TIF made *in vitro* forms stable complexes with an HSV-1 DNA fragment containing an α-TIC site and with proteins contained in a nuclear extract of mock-infected HeLa cells. Parallel *in vitro* translations of α-TIF

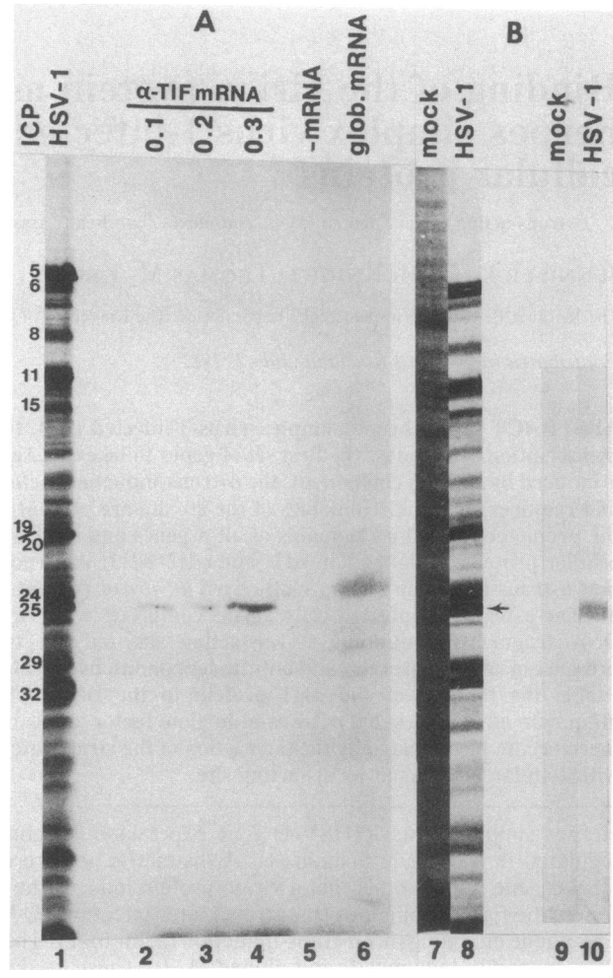

FIG. 2. Autoradiographic and photographic images of infected (HSV-1) or mock-infected (mock) cell extracts electrophoretically separated in denaturing polyacrylamide gels. Vero cells were grown to confluency in 25-cm^2 plaque dishes, mock-infected or infected with HSV-1(F) at 5 plaque-forming units/ml, and labeled with [^{35}S]methionine at 5 μCi/ml (1128 Ci/mmol, New England Nuclear) from 20 to 24 hr after infection in methionine-free 199V medium (27) (lanes 1, 7, and 8). Extracts were prepared, and proteins were electrophoretically separated on 9.3% $NaDodSO_4$/polyacrylamide gels, electrically transferred to nitrocellulose, and autoradiographed on Kodak XS film (27). SP6 transcribed α-TIF mRNA or rabbit globin mRNA was translated for 2 hr at 30°C using a rabbit reticulocyte *in vitro* translation system according to manufacturer specifications (Bethesda Research Laboratories) (lanes 2–6). [^{35}S]Methionine-labeled protein was obtained by omitting methionine from the translation mixture, pulsing the reaction for 30 min with 5–10 μCi of [^{35}S]methionine stabilized with 2-mercaptoethanol and pyridine 3,4-dicarboxylic acid [1250 Ci/mmol (Amersham)] followed by a 90-min incubation with 50 μM unlabeled methionine. The reactions were terminated by removing the samples to −20°C. Indicated for each lane is the amount of α-TIF mRNA translated in μg per reaction mixture. Rabbit globin mRNA (0.3 μg) was used (lane 6) as a positive control. Mock-infected and infected cell extracts, separated on denaturing gels as above and electrically transferred to nitrocellulose, were incubated with a 1:100 dilution of serum from a rabbit immunized with an α-TIF peptide. This 12-amino acid peptide represents the N-terminal sequences of α-TIF. It was conjugated to keyhole limpet hemocyanin, and the rabbit was booster injected with *in vitro* synthesized α-TIF (lanes 9 and 10). The blots were then immunochemically stained with anti-rabbit antibody conjugated to peroxidase (Vectastain). The details regarding the preparation of the antiserum will be described.

mRNA were done in unlabeled methionine or in [^{35}S]methionine. Two additional parallel translation reactions, one labeled and one unlabeled, were done without α-TIF mRNA,

to serve as controls for factors present in the rabbit reticulocyte extract. The *in vitro* translation mixture (5 μg) was incubated with 1 μg of the nuclear extract of mock-infected cells, 2 μg of poly(dI)·poly(dC), and ≈5 ng of unlabeled or [γ-^{32}P]ATP-end-labeled 48α27R DNA. The 48α27R DNA fragment probe (Fig. 1*A*) contains 48 bp of the regulatory domain of the HSV-1(F) α27 gene extending from position −134 to position −182 relative to the transcriptional initiation site of the gene. This fragment contains an α-TIC site, and it has been shown (12, 13) to confer α-TIF-dependent α gene regulation on α as well as non-α gene promoters. This probe has also been shown to form stable complexes with the proteins αH1 and αH2-αH3 contained in nuclear extracts of mock-infected cells (14).

The results of the gel retardation assays done in synthetic poly(dI)·poly(dC) homopolymer competitor DNA and mock-infected cell extract (see Fig. 3*A*) show the following. The addition of α-TIF to the reaction mixture (lane 3) results in the appearance of a protein–DNA complex forming two closely migrating bands with an electrophoretic mobility slower than the complexes in nuclear extracts of mock-infected cells alone (lane 1), or in nuclear extract mixed with unlabeled control rabbit reticulocyte extract (lane 2).

Further evidence that this complex contains α-TIF is demonstrated in lanes 4 and 5. The complexes resolved in these lanes were identical to those shown in lanes 2 and 3, except that the 48α27R DNA was unlabeled, and the α-TIF added was synthesized in [^{35}S]methionine. The significant feature of these results is the presence of ^{35}S-labeled doublet bands in lane 4 that comigrated with the doublet bands seen in lane 3. Since the only major ^{35}S-labeled protein synthesized *in vitro* is α-TIF (Fig. 2*A*), it can be concluded that the protein–DNA complex contains α-TIF. In the experiments described below, this complex is designated as the α-TIF–DNA complex.

The αTIF–DNA Complex Is Also Formed in Infected Cell Extracts. In contrast to the results obtained with extracts of mock-infected cells, the extracts of nuclei of infected cells do not require exogenously added α-TIF to form the α-TIF–DNA complexes described above (Fig. 3*B*, lane 7). To verify that α-TIF was present in both of the complexes identified as α-TIF–DNA, the DNA binding reactions were done in the polyclonal antibody against the α-TIF synthetic peptide. The design of this experiment was based on the observation that antibody reacting specifically with a DNA binding protein can further retard the migration of the DNA–protein complex in retardation gels (17, 28). As shown in Fig. 3*C*, addition of the immune serum retarded the migration of the α-TIF–DNA complexes formed in nuclear extracts of mock-infected cells supplemented with the *in vitro* synthesized α-TIF (lane 13) and in nuclear extracts of infected cells (lane 14), whereas the same amount of preimmune serum had no effect (lanes 11 and 12). The results conclusively prove the presence of α-TIF in these DNA complexes.

Formation of the α-TIF–DNA Complex Requires Proteins in Nuclear Extracts of Mock-Infected Cells. In the experiments summarized above, we demonstrated that the α-TIF–48α27R DNA complexes formed by nuclear extracts of infected and of mock-infected cells supplemented with α-TIF synthesized *in vitro* cannot be differentiated with respect to electrophoretic mobility and reactivity with the polyclonal rabbit antibody. In the experiments described below, we demonstrate that the formation of the complex depends on the availability of at least one nuclear protein with a binding site in the cis-acting site for α-TIF.

The results of the first series of experiments (Fig. 4) indicate the following. (*i*) The formation of the α-TIF–DNA complex required the nuclear extract of mock-infected cells supplemented with the α-TIF made *in vitro* (lanes 2–4) or of the nuclear extract of infected cells in the absence of added

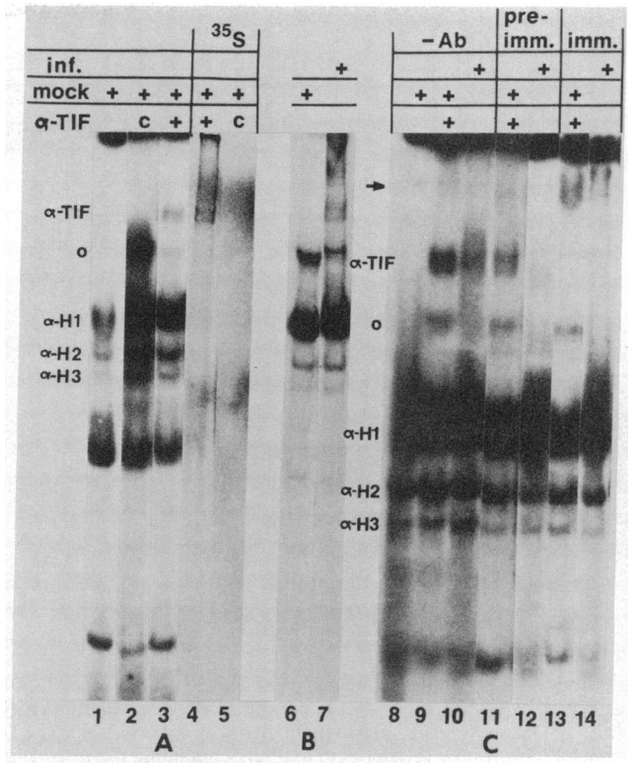

FIG. 3. Autoradiographic images of labeled protein–48α27R DNA fragment complexes separated in 4% nondenaturing polyacrylamide gels. The gel retardation assays were done as described (14, 18, 19). (*A*) Mock-infected HeLa cell nuclear extract (1 μg) was incubated with 5 ng of 48α27R DNA and 2 μg of poly(dI)·poly(dC) (Pharmacia P-L Biochemicals). In addition, 5 μg of control (c) or reticulocyte extract containing the *in vitro* translated α-TIF (+) were included where indicated. The samples in lanes 1–3 contained ^{32}P-labeled 48α27R DNA fragment and unlabeled reticulocyte extracts. The samples in lanes 4 and 5 contained unlabeled DNA and ^{35}S-labeled α-TIF. (*B*) Mock-infected (mock) or 12-hr infected (inf.) nuclear extracts (3 μg) were incubated with 2 ng of labeled 48α27R DNA fragment with 3 μg of poly(dI)·poly(dC). (*C*) Mock-infected (mock) or 12-hr infected (inf.) extract (1 μg) was incubated with labeled 48α27R DNA and 2 μg of poly(dI)·poly(dC). The reaction mixtures lacked antibody (−Ab) or contained 2 μl of a 1:6.7 dilution of either the preimmune (pre-imm.) or immune (imm.) rabbit serum (Fig. 2). The protein concentrations, in a final volume of 15 μl, were maintained at 15 μg per reaction mixture by the addition of bovine serum albumin. Open circles, αH1+αH2-αH3 complex.

α-TIF (lanes 11–13). Exogenously added α-TIF, made *in vitro*, increased the amount of the α-TIF–DNA complex formed in the nuclear extract of infected cells alone (lanes 8–13). (*ii*) The synthetic deoxy polymer used as the competitor DNA in the experiments illustrated in Figs. 3 and 4*A* has been shown to compete with the labeled DNA fragment for the binding of the αH2-αH3 proteins but not for the binding of the αH1 proteins (ref. 14; T.M.K. and B.R., unpublished results). The evidence that αH2-αH3 proteins may not be involved in the formation of the α-TIF–DNA complex is underscored by the observation that the α-TIF–DNA complex did not form under any conditions tested with the heteropolymer poly(dA-dT)·poly(dA-dT) as the competitor DNA (Fig. 4*B*, lanes 1–19). This synthetic oligomer competes with the 48α27R DNA probe for αH1 protein but does not affect the binding of αH2-αH3 protein to its cognate DNA sequence (ref. 14; T.M.K. and B.R., unpublished results).

The purpose of the second series of experiments (Fig. 5) was to define the role of the αH1 and αH2-αH3 proteins in the formation of the α-TIF–DNA complex. The experimental design and the salient features of the results were as follows.

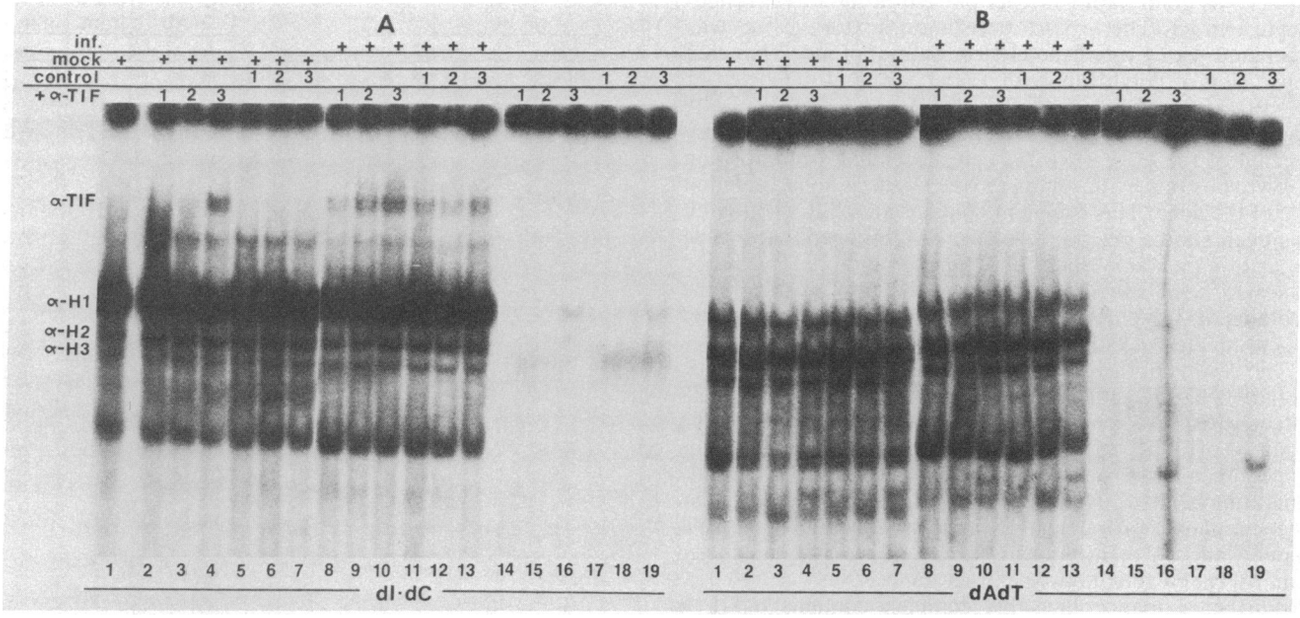

FIG. 4. Autoradiographic images of labeled protein–48α27R DNA complexes in the presence and absence of nuclear factors, comparing the effects of two synthetic DNA competitors. The gel retardation assays were done as described in Fig. 3. (*A*) Mock-infected (mock) or 12-hr infected (inf.) nuclear extract (1 μg) was incubated with 5 ng of labeled 48α27R DNA fragment and 3 μg of poly(dI)·poly(dC) (lanes 1–13). Where indicated, control (control) or reticulocyte extracts containing the *in vitro*-translated α-TIF (α-TIF) were included in the following concentrations: lane 1, 0.25 μg; lane 2, 1 μg; and lane 3, 5 μg. Labeled 48α27R DNA fragment (5 ng) and 3 μg of poly(dI)·poly(dC) were incubated with the indicated amounts of control or α-TIF-containing reticulocyte extracts in the absence of nuclear extracts (lanes 14–19). (*B*) Lanes 1–19 are identical to those in *A*, except that poly(dI)·poly(dC) was replaced with 3 μg of poly(dA-dT)·poly(dA-dT) (Pharmacia P-L Biochemicals) as the competitor DNA. The protein concentrations were maintained at 6 μg per reaction mixture.

(*i*) Previous studies demonstrated that the 29-bp fragment from the regulatory domain of the α0 gene (29α0R) bound the αH1 proteins but not the αH2-αH3 proteins, although it competed for the latter proteins with the 48α27R fragment (ref. 14; T.M.K. and B.R., unpublished results). The results shown in lanes 1–9 indicate that the 29α0R fragment formed a complex with proteins that included α-TIF with nuclear extract of mock-infected cells and poly(dI)·poly(dC), but not with poly(dA-dT)·poly(dA-dT) competitor or in the absence of α-TIF. (*ii*) αH1 and αH2-αH3 were separated by several chromatographic procedures and were shown to bind both independently (Fig. 5, lanes 12–21) and, in reconstituted mixtures, concurrently (Fig. 5, lanes 22–26) to the α-TIC domains of the α27 gene (48α27R) (T.M.K. and B.R., unpublished results). The DNA band shift assays done with the DEAE-Sepharose fractions containing the separated αH1 and αH2-αH3 proteins show that the α-TIF–DNA complexes were formed only with the synthetic competitor DNA that did not block the binding of αH1 to its cognate DNA sequence (Fig. 5, lane 14). The chromatographic fractions containing

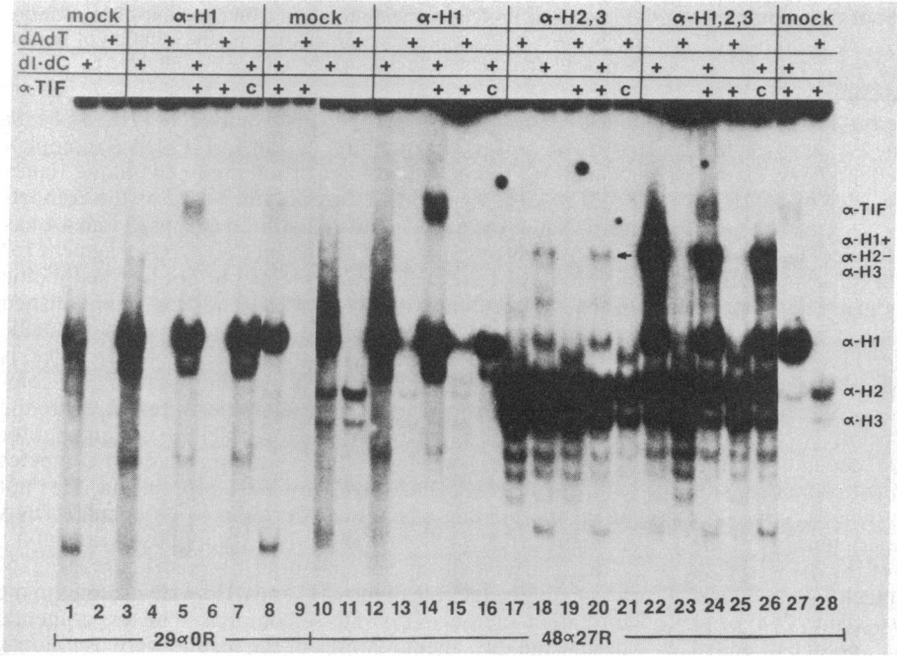

FIG. 5. Autoradiographic images of protein–DNA complexes formed by 48α27R and the 29α0R DNA fragments with various competitor DNAs. The assays were done as described in Fig. 4, using 1.5 or 5 ng of labeled 29α0R or 48α27R DNA fragments, respectively; 3 μg of poly(dI)·poly(dC) (dI·dC) or poly(dA-dT)·poly(dA-dT) (dAdT) competitor DNA; 1 μg of mock-infected (mock), 2 μg of the DEAE-Sepharose fraction 4 (αH1), or 6.2 μg of fraction 22 (αH2-αH3) prepared as described (T.M.K. and B.R., unpublished results); and 10 μg control (c) or reticulocyte extract containing the *in vitro* translated α-TIF (+), as indicated. The concentrations of the reconstituted mixtures (αH1, αH2-αH3) were additive. Protein concentrations were maintained at 19 μg per reaction mixture.

αH2-αH3 did not preclude the formation of the α-TIF–DNA complexes even under conditions in which concurrent binding of αH1 and αH2-αH3 was demonstrable (lane 24).

DISCUSSION

The virion component (α-TIF)-mediated trans-induction of α genes requires a specific cis-acting site within the promoter-regulatory domains of these genes (9–13). Identification of this α-TIC site was based on construction of chimeric genes consisting of regions of the α promoter-regulatory domains fused to HSV-1 promoters (9–13). These studies showed that, whereas the 29-bp fragment from the α0 gene (29α0R) conferred α-gene regulation on an α-promoter-driven gene, the 48-bp fragment from the α27 gene (48α27R) conferred α-gene regulation on both α- and non-α-gene promoters (12, 13). Both fragments contain a homolog of the sequence 5′ GYATGNTAATGARATTCYTTGNGGG 3′, where Y is a pyrimidine, R is a purine, and N is any nucleotide, present in the promoter-regulatory domains of all α genes (9). Subsequent studies showed that the homologs bind two host proteins, αH1 and αH2-αH3 (14). The αH1 protein protects the α-TIC sequence from DNase I digestion, and the purine methylation interference studies suggest that it binds primarily to the 5′ domain of this sequence. The αH2-αH3 protects an overlapping 3′ domain of the α-TIC site and extends further in the 3′ direction (ref. 14; T.M.K. and B.R., unpublished results). The 29α0R fragment does not contain the entire αH2-αH3 binding site, and, whereas it competes with other homologs for the protein, its binding to the αH2-αH3 protein appears to be unstable. Both proteins bind to the 48α27R fragment. The central question that remained unresolved was the interaction of α-TIF with the cellular proteins that interact with the α-TIC site.

In this paper we report two significant findings. First, α-TIF stably binds to DNA–protein complexes. This conclusion rests on two observations. First, [^{35}S]methionine-labeled α-TIF synthesized *in vitro* bound to DNA–protein complexes that comigrated with the complexes formed by labeled DNA fragments and nuclear extracts of infected, but not of uninfected, cells. Second, antibody made to a synthetic peptide, whose sequence had been predicted by the nucleotide sequence of the α-TIF gene, reacted with and retarded the electrophoretic mobility of the DNA complexes containing α-TIF. The second significant observation is that the presence of α-TIF in DNA–protein complexes appears to depend on conditions that support the binding of αH1 to the DNA rather than on the mere presence of this cellular protein in the reaction mixture. In this instance, conditions that precluded the binding of αH1 also precluded the formation of DNA–protein complexes containing α-TIF. Specifically, the heteropolymer poly(dA-dT)·poly(dA-dT) that competes with α-TIC sites for the binding of αH1 also precluded the formation of the α-TIF–DNA complex. Whereas the chromatographic fractions containing αH1 resulted in the formation of α-TIF–DNA complexes, those containing αH2-αH3 did not. The participation of αH2-αH3 in the formation of the αTIF–DNA complex also appears to be excluded by the observation that the 29α0R fragment that binds αH1 but not αH2-αH3 was able to form complexes with α-TIF under conditions that support the binding of αH1 protein. Notwithstanding these findings, the definitive evidence that αH1 forms a complex with α-TIF and the α-TIC sites in promoter-regulatory domains of α genes must await studies with purified proteins.

The results presented in this report indicate that the induction of α genes by α-TIF is mediated by the interaction of α-TIF with host proteins binding at the α-TIC site. The central question that remains to be resolved is the mechanism by which α-TIF alters the DNA–protein interactions at the α-TIC sites to enable the induction of α-gene expression. Reports from this laboratory have shown that homologs of the α-TIC sequence are present in promoter domains of both inducible and constitutively expressed genes (29) and that the constitutive level of expression of the chimeric genes with and without the α-TIC sequence is not significantly different (12, 13). Although there is no definitive evidence to discriminate between the hypotheses that α-TIF alleviates the suppression of α gene expression by host factors and that it acts positively to mobilize host transcriptional factors at the α-TIC site, the evidence that α-TIF interacts with its cis site reported in this paper represents a significant step toward understanding the function of this distinctive virion component.

These studies were aided by U.S. Public Health Service Grants CA08494 and CA19264 from the National Cancer Institute and AI124009 from the National Institute of Allergy and Infectious Diseases and by the Grant MV-2W from the American Cancer Society. J.L.C.McK. and T.M.K. were trainees supported by the U.S. Public Health Service Training Grant CA09241 from the National Cancer Institute.

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