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Anti-OTF-1 antibodies inhibit NFIII stimulation of *in vitro* adenovirus DNA replication

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ABSTRACT

HeLa cell OTF-1 has been purified on the basis of its DNA binding activity and used to raise a polyclonal rabbit antiserum. This antiserum is shown to recognize both native and denatured OTF-1 from both human and a similar protein from Xenopus culture cells, but to react either more weakly or not at all with the lymphoid cell-specific OTF-2. Separately, NFIII has been purified on the basis of its ability to stimulate Adenovirus DNA replication in vitro. On denaturing polyacrylamide gels OTF-1 and NFIII exhibit identical mobility. Anti-OTF-1 antiserum recognizes NFIII and neutralizes its stimulatory effect on DNA replication. Moreover, OTF-1 can functionally replace NFIII. Taken together with previously published DNA binding data, this indicates that OTF-1 and NFIII are either very closely related or identical.

INTRODUCTION

The octamer motif, ATGCAAAT, was first noted as a sequence conserved in immunoglobulin gene promoters (1, 2). It has since been implicated in the expression of a variety of different types of gene including UsnRNA genes (3, 4, 5, 6), lymphoid cell-specific genes (1, 2) histone H2b genes (7, 8) and viral genes (9, 10, 11). A seemingly ubiquitous protein which specifically binds this sequence was identified (12) and shown to bind to a variety of promoters and enhancers containing the octamer motif and variants thereof (13, 14, 15). More detailed study revealed the presence of two different proteins, one lymphoid cell specific, the other ubiquitous, whose DNA binding properties were indistinguishable (16, 17, 18, 19, 20, 21) and suggested that there are possibly even more than two octamer binding proteins (22).

Recently two octamer binding proteins have been purified (20, 21, 23). The ubiquitous protein, purified from HeLa cells and

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renamed OTF-1 (octamer-binding transcription factor-1) was shown to stimulate transcription from a human histone H2b promoter in vitro (20). The lymphoid specific OTF-2 was purified from Namalwa cells and stimulated transcription from an Immunoglobulin Kappa light chain promoter in vitro (21). We have chosen to adopt the OTF-1/OTF-2 nomenclature for this publication, although these activities have been given several different names.

NFIII (nuclear factor III) was first identified as a cellular activity required for optimal adenovirus DNA replication in vitro (24, 25). In adenovirus serotype 2 (Ad2), where its activity was first noted, it was shown to act through the sequence TATGATAATGAG, which contains a 6 out of 8 match to the octamer motif. Careful analysis of the binding specificity of purified NFIII revealed that its preferred binding site contains ATGCAAAT (26, 27, 28). These data suggested that OTF-1 and NFIII might be related. Here we show that purified OTF-1 and NFIII exhibit identical electrophoretic mobilities and use an antiserum raised against purified OTF-1 to show cross-reaction with pure NFIII. Furthermore, the anti-OTF1 antiserum inhibits NFIII stimulation of adenovirus DNA replication and purified OTF1 can functionally replace NFIII in this system. Taken together these data indicate that OTF-1 and NFIII are probably identical.

MATERIALS AND METHODS

Purification of OTF-1

HeLa nuclear extract was prepared by either the method of Dignam et al. (29) or Wildeman et al. (30). 50-100 mg nuclear protein in C buffer (20 mM Hepes pH 7.9, 0.2 mM EDTA, 0.5 mM DTT, 2 mM $MgCl_2$, 0.5 mM PMSF) containing 100 mM KCl was passed over DEAE-Sephadex (A25). The flow through fraction was collected, diluted to 50 or 75 mM KCl and loaded onto Heparin Sepharose. OTF-1 bound and was eluted with a 75-600 mM KCl gradient. OTF-1 eluted in a broad peak between 175 and 350 mM KCl. These fractions were collected, diluted to 100 mM salt, made 0.1% in Nonidet P-40, and loaded onto an oligonucleotide affinity column. Either of two columns was used. The first utilised a concatemerised 36-mer sequence derived from the U2 DSE (31) and was prepared by the method of Kadonaga and Tjian (32). The second

was prepared identically to that of Fletcher et al. (20). Poly(dI-dC).poly(dI-dC) was included in the affinity column loading buffer (32). OTF-1 was eluted either with a 0.1-1.0 M KCl gradient or with a 1.0 M KCl step. OTF-1 binding activity was assayed either by gel retardation assays (33, 34) or by DNaseI footprinting (35). Purity was assayed by silver staining of SDS-polyacrylamide gels on which aliquots of the fractions were loaded. Three consecutive passages over the affinity column were sufficient to obtain a high degree of purity.

Protein gels, immunodecoration

SDS-polyacrylamide gels were prepared according to Laemmli (36). 7.5% or 10% gels were routinely used. Proteins were transferred to nitrocellulose by electroblotting (37). The electroblot was then incubated either with pre-immune or immune serum (diluted 1:50), washed and incubated with goat anti-rabbit IgG coupled to alkaline phosphatase (Promega). Bound antibody was revealed by a colour reaction utilising nitro blue tetrazolium and 5-bromo-4-chlor-3-indolyl phosphate (Promega) as substrates.

Indirect immunoprecipitation and gel retardation assays

Gel retardation assays were carried out as described (15, 26). Where indicated antisera were included in the pre-incubation step. For indirect immunoprecipitation binding reactions identical to those used for gel retardation assays were carried out. These were then transferred onto protein A-Sepharose beads (Pharmacia) to which anti-OTF-1 had been coupled (coupling conditions from Mattaj and De Robertis (38). After 15 min on ice these mixtures were diluted to 1 ml with 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1% Nonidet P-40. The beads were then pelleted and resuspended three times in 1 ml of the same buffer to wash away unbound material. DNA was extracted from the beads as described for RNA (38) and loaded onto a 15% polyacrylamide gel. After electrophoresis the gel was dried and autoradiographed.

Purification of NFIII

A HeLa cell nuclear extract was prepared as described previously (24) and passed twice over DEAE-cellulose, firstly at 200 mM NaCl in buffer A (25 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF, 20% glycerol) to free it from nucleic acids and secondly, after dialysis, at 30 mM NaCl in

buffer B (25 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF, 20% glycerol, 10% sucrose, 0.01% Nonidet P40). The flow-through and first wash fractions (buffer B, 30 mM NaCl) were combined and applied to a phosphocellulose column. Bound proteins were eluted with a linear 0.03-1 M NaCl gradient (buffer B). NFIII eluted at 0.13 M NaCl. NFIII containing fractions were combined and applied to a pKB67-88 DNA-cellulose column, a sequence-specific DNA affinity resin (39). Bound proteins were eluted with a linear 0.1-1 M NaCl gradient (buffer B). NFIII eluted at 0.35 M NaCl. Finally the active fractions, diluted to 0.1 M NaCl, were chromatographed on a denatured calf thymus DNA-cellulose column, which was eluted stepwise with 0.35 M and 1 M NaCl in buffer B. NFIII eluted with the 0.35 M NaCl step.

During the entire purification procedure NFIII was monitored by assaying its stimulation of adenovirus DNA replication in vitro. In the flow-through of the second DEAE-cellulose column no activity was observed due to the presence of an inhibitor(s) which masked NFIII activity. This inhibitory activity was separated from NFIII on the phosphocellulose column, from which it eluted at 0.4 M NaCl. The NFIII specific DNA-binding activity (27) copurified with the DNA replication stimulatory activity.

Elution of polypeptides from SDS-polyacrylamide gels and protein renaturation were performed exactly as described by Hager and Burgess (40).

Adenovirus DNA replication in vitro

Adenovirus DNA replication was studied in a reconstituted system essentially as described using either XhoI-digested adenovirus 5 DNA-terminal protein complex (41) or EcoRI- and NdeI-digested plasmids pEI0III and pEI (containing an adenovirus origin with or without a functional NFIII binding site respectively; the construction of pEI0III and pEI will be described elsewhere) (27) as template. The adenovirus 5 precursor terminal protein-DNA polymerase complex was isolated from recombinant vaccinia virus infected cells (42). Sera were preheated for 60 min at 60°C before addition to NFIII in order to remove non-specific inhibitors. Non-immune rabbit serum was added to all reactions to give a constant amount of serum (3 µl) per reaction. Sera were preincubated with NFIII for 30 min on ice.

RESULTS

Purification of OTF-1

OTF-1 was purified from HeLa cell nuclear extract (29, 30) using a combination of conventional and sequence-specific DNA affinity chromatographic steps (see Materials and Methods for details). The results of one purification are shown in the silver stained protein gel in Figure 1. In this preparation an octamer motif-containing oligonucleotide affinity column constructed according to the method of Kadonaga and Tjian (32) was used. After 3 passages over the column a highly purified protein of roughly 100 kD was obtained. The peak fractions of the third passage, whose activity was monitored by a DNaseI footprinting assay (35) are shown in lanes 9-12 of Figure 1. The size of the protein agrees with the size estimated by excision of protein bands from SDS-polyacrylamide gels, protein renaturation and gel retardation assay (data not shown) and with the value published by Sturm et al. (23), but is slightly greater than that published by Fletcher et al. (20) for OTF-1 and by O'Neill and Kelly (43) for NFIII. We attribute these differences either to differences

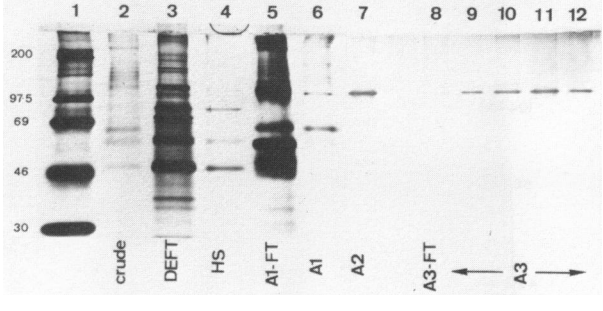


Figure 1. Purification of OTF-1.
A silver-stained 7.5% SDS-polyacrylamide gel showing the protein profile of various stages of the purification. Lane 1: Protein size standards. Lane 2: Crude nuclear extract. Lane 3: 100 mM Flow-through from the DEAESephadex column. Lane 4: Pooled active Heparin-Sepharose step fractions. Lane 5: Flow-through of the first affinity column passage. Lane 6: Pooled active fractions from the first affinity column passage. Lane 7: Pooled active fractions from the second affinity column passage. Lane 8: Flowthrough of the third affinity column passage. Lanes 9-12: Peak fractions of the third affinity column passage. OTF-1 was monitored by DNAseI footprinting assays using the U2 DSE DNa substrate (31).

in electrophoresis conditions or to slight proteolytic degradation since we obtained the same size of protein using an oligonucleotide affinity column identical to that of Fletcher et al. (20) (see also Fig. 5c).

Anti-OTF-1 antiserum recognizes both the native and denatured protein

The material (roughly 10 µg protein) from the affinity column was used directly for antibody production by injection into rabbit lymph nodes (44). After several booster injections an antiserum was obtained which recognized pure OTF-1 (Fig. 2B, lanes 1 and 2) as well as a protein of the same size in crude nuclear extract or whole cell extract (Fig. 2B, lanes 4 and 5). Additionally two other bands of roughly 65 kD were decorated by both pre-immune and immune serum in whole cell extract (Figs. 2A and B, lane 5) and a single band of roughly 55 kD was seen in

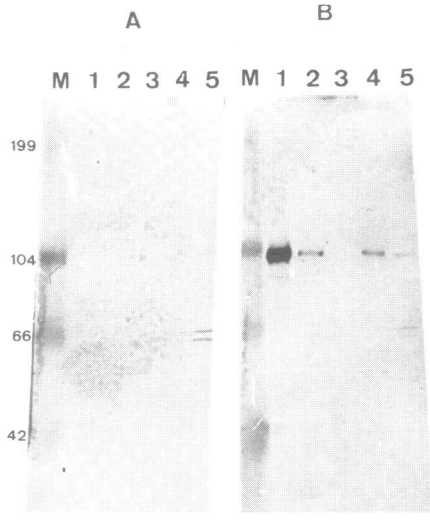


Figure 2. Immunodecoration with anti-OTF-1.
OTF-1 antiserum (B) and pre-immune serum (A) were used to stain nitrocellulose filters to which pure OTF-1, crude HeLa nuclear extract or whole cell extract had been transferred after fractionation on a 7.5% SDS-polyacrylamide gel. M:Protein size markers (pre-stained with Coomassie Blue). Lanes 1,2,3: 100, 10 and 2 µl aliquots of pure OTF-1. Lane 4: 30 µg of crude nuclear protein extract. Lane 5: 30 µg of whole cell extract. The bound antibody was visualised with goat anti-rabbit IgG coupled to alkaline phosphatase.

nuclear extract which was only recognized by immune serum (Fig. 2B, lane 4). This protein co-fractionated with mitochondria which contaminate the crude nuclear preparation from which the nuclear extract is prepared (data not shown).

To determine whether the antiserum recognized native OTF-1 we carried out an indirect immunoprecipitation assay (45). Two different double stranded 40-mer oligonucleotides which both contained octamer motifs, originating from the U2 gene DSE and the immunoglobulin heavy chain gene enhancer (see ref. 15 for sequences), were used. The control was a double-stranded 30-mer which did not contain an octamer motif. The oligonucleotides were end-labelled and an equal mixture of one of the 40-mers and the 30-mer was incubated with crude nuclear extract under conditions previously used for gel retardation experiments (15). This allowed specific association of the oligonucleotide with its cognate binding factor(s). Then complexes including OTF-1 were specifically immunoprecipitated using anti-OTF-1 bound to protein A-Sepharose. After washing, DNA was extracted and analysed. Fig. 3, lanes 1 and 4 show the total DNA input with the immunoglobulin

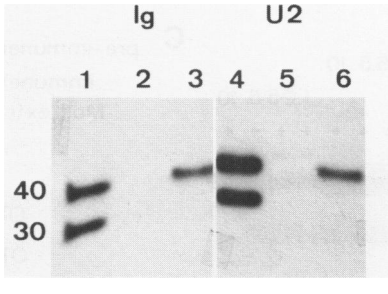
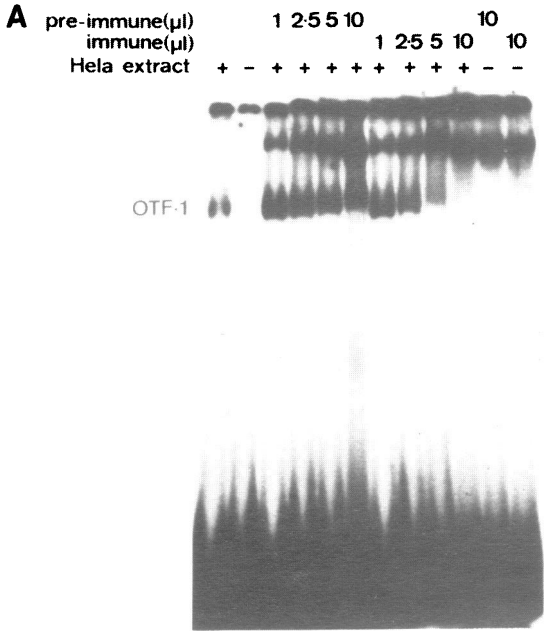


Figure 3. Indirect immunoprecipitation of octamer-containing oligonucleotides with anti-OTF-1.

Two double-stranded 40-mer oligonucleotides which contained the octamer motif, one containing U2 promoter sequences, the other Immunoglobulin heavy chain enhancer sequences (Ig) (15), and a double stranded 30-mer lacking the octamer motif were used. The oligonucleotides were end-labelled by polynucleotide kinase, and equal amounts of one of the 40 mers plus the 30 mer were incubated in crude HeLa nuclear extract, then immunoprecipitated with anti-OTF-1. DNA was extracted from the immunoprecipitates and fractionated on a 15% polyacrylamide gel. The gel was dried and autoradiographed. Lanes 1-3: Ig oligo, lanes 4-6: U2 oligo. Lanes 1 and 4: input DNA. Lanes 2 and 5: immunoprecipitation with pre-immune serum. Lanes 3 and 6: immunoprecipitation with anti-OTF-1.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Hela extract
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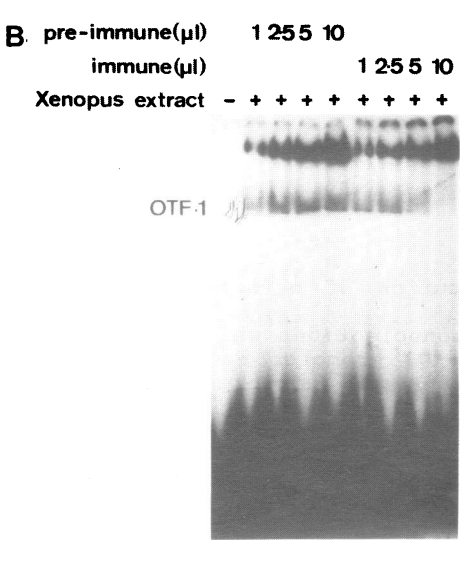
10
OTF·1


B
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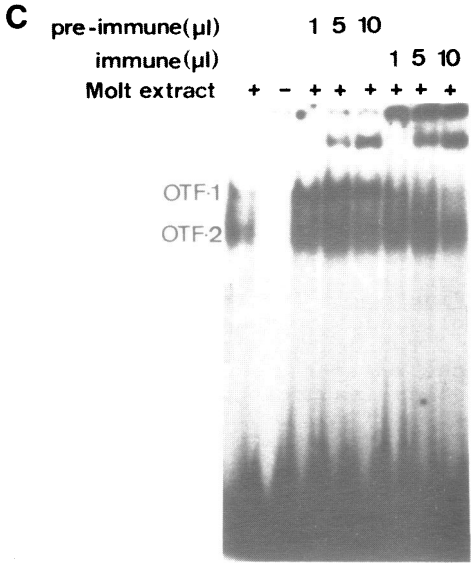
Xenopus extract
1 2·5 5 10
1 2·5 5 10
OTF·1


C
pre-immune(µl)
immune(µl)
Molt extract
1 5 10
1 5 10
OTF·1
OTF·2

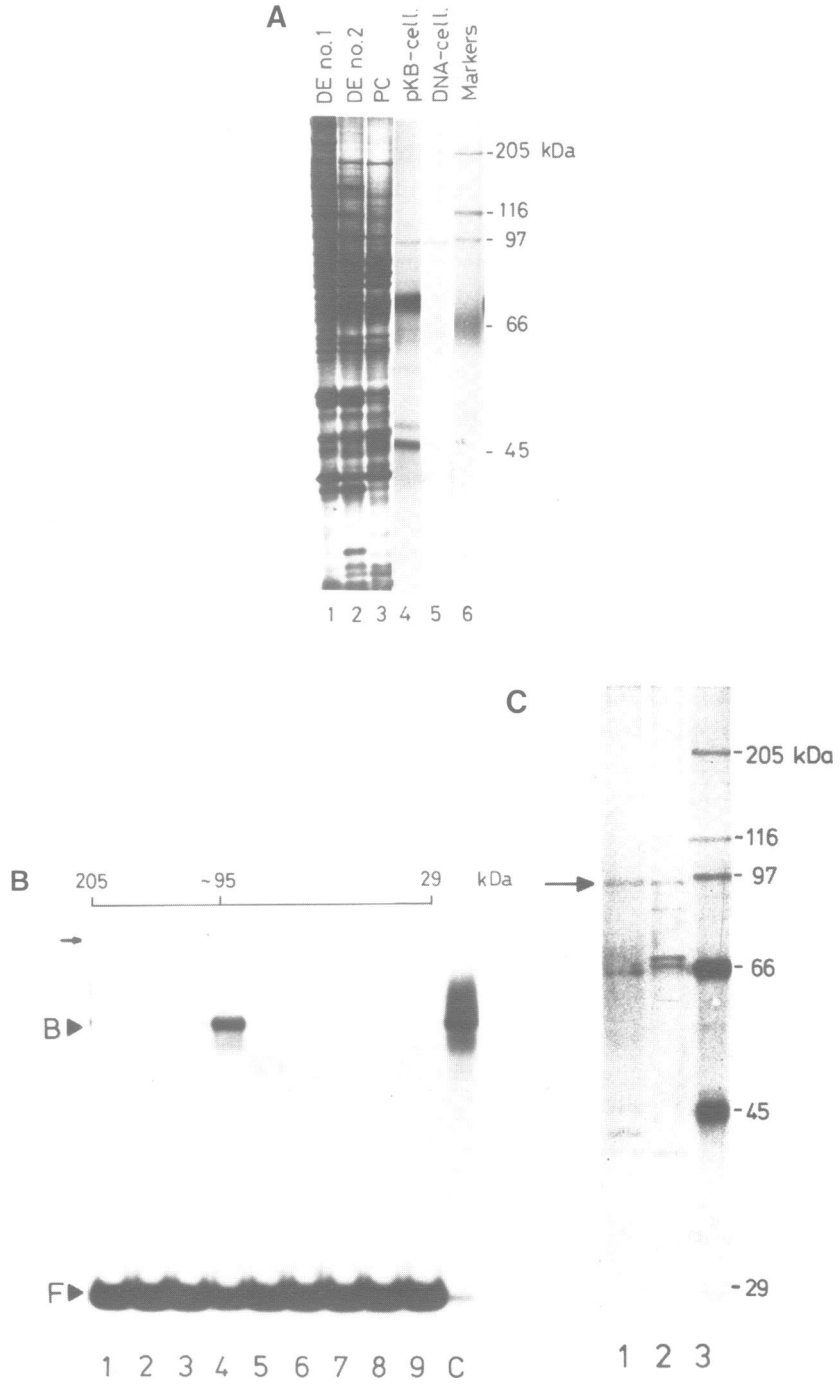
and U2 oligonucleotides respectively. Lanes 2 and 5 are the pre-immune serum controls. Specific precipitation of the octamer-containing oligonucleotides (lanes 3 and 6) was observed, demonstrating that the antiserum recognized native OTF-1. By preincubation of nuclear extract with antibody prior to oligonucleotide addition we failed to interfere with specific precipitation, indicating that the antiserum did not contain a high titer of antibodies against the DNA binding domain of OTF-1.

Anti-OTF-1 recognizes Xenopus OTF-1 but reacts more weakly or not at all with human OTF-2

In order to analyse the relationship of HeLa OTF-1 to similar activities in other species (14, 31, 46) and to the lymphoid-specific OTF-2 (16, 17, 21) we determined whether anti-OTF-1 cross-reacted with these proteins. To do this we utilised a gel retardation assay. Incubation of the immunoglobulin 40-mer in crude nuclear extract results in a characteristic retarded band, attributable to OTF-1 binding (12, 15). This is seen in Fig. 4A, lane 1. Incubation of either pre-immune or immune serum with probe also results in the production of a retarded complex (Fig. 4A, last two lanes). While inclusion of up to 10 µl of pre-immune serum in the gel retardation assay does not affect the OTF-1 complex, addition of more than 5 µl of immune serum results in disappearance of complex (Fig. 4A, lanes 3-10). Repeating the same assay with crude extract prepared from nuclei of Xenopus cultured kidney cells gives a similar result (Fig. 4B), showing that anti-HeLa

Figure 4. Interaction of anti-OTF-1 with octamer binding proteins from different cell types.

Gel retardation assays were carried out as previously described (15) with a double-stranded 40-mer oligonucleotide from the Immunoglobulin heavy chain gene enhancer containing an octamer motif, except that anti-OTF-1 or pre-immune serum was included during the preincubation of extract with non-specific DNA carrier before probe addition. Retarded DNA complexes corresponding to the binding of OTF-1 or OTF-2 are indicated. Panel A. HeLa cell nuclear extract. Panel B. Xenopus kidney cell extract. Panel C. Molt-4 cell nuclear extract. Conditions were chosen such that free probe was always in large excess to that there was no competition between octamer binding proteins and serum DNA binding proteins.


A
DE no.1
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OTF-1 recognizes a related Xenopus protein, presumably Xenopus OTF-1.

When lymphoid cell nuclear extracts are used in a similar experiment two distinct complexes appear which are due to the binding of the specific octamer motif-binding proteins OTF-1 and OTF-2 (16, 17, 21). In Fig. 4C an extract from Molt-4 cells, a human T cell line (47), was used. Complexes attributable to OTF-1 and OTF-2 are indicated. Anti-OTF-1 results in the disappearance of the OTF-1 complex, but does not affect the OTF-2 complex more than does pre-immune serum (Fig. 4C, compare lanes 5 and 8). With higher amounts of either immune or preimmune serum complex formation is non-specifically inhibited. These results indicate that HeLa OTF-1 is more closely antigenically related to Xenopus OTF-1 than to Molt-4 OTF-2, and that anti-OTF-1 does not interact significantly with OTF-2 at these serum concentrations, in spite of the fact that the DNA recognition specificity of these two proteins is identical.

Purification of NFIII

NFIII was purified by a combination of conventional ion-exchange and sequence-specific DNA affinity column chromatographic methods. The crucial step of this procedure is affinity chromatography on a column containing multiple copies

Figure 5. Purification of NFIII.
A. A silver-stained 10% SDS-polyacrylamide gel showing the protein profile of various stages of the purification. Lane 1: 200 mM Flow-through of the first DEAE-cellulose column. Lane 2: 30 mM Flow-through of the second DEAE-cellulose column. Lane 3: Pooled active fractions from the phosphocellulose column. Lane 4: Pooled active fractions from the pKB67-88 DNA-cellulose column. Lane 5: 0.35 M NaCl step fraction from the nonspecific denatured DNA-cellulose column. Lane 6: Protein size standards. B. A 20 µl sample of the nonspecific DNA-cellulose step fraction was electrophoresed on an 8.75% SDS-polyacrylamide gel. Proteins from individual gel slices were eluted, renatured and assayed for DNA binding activity. Lane C: gel retardation (26) of 0.2 µl of nonspecific DNA-cellulose step fraction. Lanes 1-9: gel retardation of 5 µl of each gel slice eluate (total volume: 500 µl). The position of the NFIII-DNA complex in the gel is indicated.
C. Comparison of the electrophoretic mobilities of OTF-1 and NFIII. OTF-1 and NFIII preparations from late steps in purification (OTF-1: second affinity column fraction; NFIII: pKB67-88 DNA-cellulose column fraction) were electrophoresed on a 10% SDS-polyacrylamide gel and visualized by silver staining. Lane 1: OTF-1. Lane 2: NFIII. Lane 3: Protein size standards. The arrow indicates the OTF-1/NFIII polypeptide.

of the adenovirus 2 origin of DNA replication (prepared from the plasmid pKB67-88, which contains 88 copies of the adenovirus origin of replication; 39). During the entire purification NFIII activity was monitored by measuring stimulation of adenovirus DNA replication in vitro (24). Purified NFIII runs on denaturing protein gels as a protein of approximately 95 kD (Fig. 5A). Octamer-specific DNA-binding activity copurified with the DNA replication stimulatory activity. After elution from a SDS-polyacrylamide gel and renaturation (40) the 95 kD polypeptide was shown to be responsible for the specific DNA-binding activity using the gel retardation assay (Fig. 5B). Because of the slight deviations in size of the protein from reported values for NFIII (43) and OTF-1 (20, 23) and from the observed value for OTF-1 (see Figs. 1 and 2) we investigated the possibility that they were due to differences in electrophoresis conditions.

In order to compare the electrophoretic mobilities of OTF-1 and NFIII directly on a denaturing protein gel partially purified preparations of OTF-1 (second affinity column fraction) and NFIII (pKB67-88 DNA-cellulose column fraction) were run under the same conditions on the same gel. The result is shown in Figure 5C and confirms that OTF-1 and NFIII consist of polypeptides with identical mobilities.

Anti-OTF-1 recognizes NFIII

The physical similarity of NFIII and OTF-1 as well as the DNA binding specificities of both proteins (15, 20, 26, 27, 43) suggest that OTF-1 and NFIII are closely related. To investigate this possibility the cross-reaction of the anti-OTF-1 antiserum with purified NFIII was analysed. Both Western blots (not shown) and gel retardation assays (Fig. 6) showed that purified NFIII is recognized by the anti-OTF-1 antiserum. In contrast to Fig. 4 no complex due to serum DNA-binding proteins was observed, because less antiserum was required when interaction between purified NFIII and the DNA probe was analysed. This allows the resolution of a second retarded band of lower mobility than the NFIII-DNA complex (Fig. 6 lanes 2-4) which we interpret as an anti-OTF-1-NFIII-DNA complex.

In order to provide further evidence that OTF-1 and NFIII are

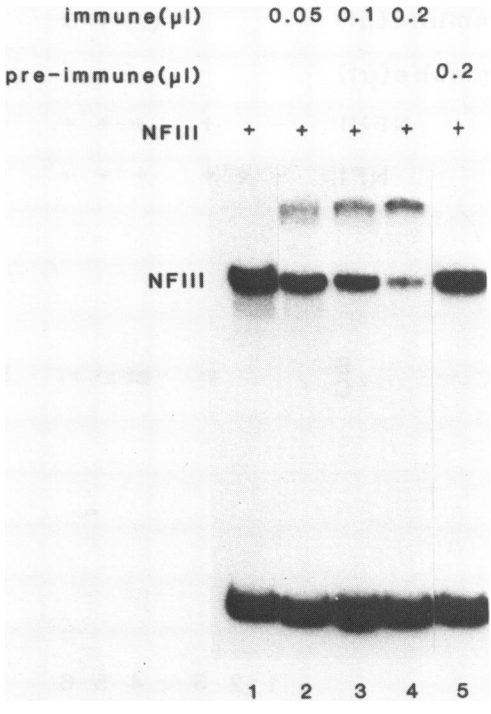

Figure 6. Interaction of anti-OTF-1 with NFIII.
Gel retardation assays were carried out as described (26) with a DNA fragment containing the adenovirus 4 replication origin. Anti-OTF-1 or pre-immune serum was preincubated with NFIII before probe addition. The position of NFIII-DNA complexes are indicated. The slower migrating complex is assumed to consist of anti-OTF1, NFIII and DNA.

identical we wished to determine whether anti-OTF-1 antiserum would inhibit the activity of NFIII. This assay is shown in Fig. 7. Adenovirus DNA replication is stimulated by addition of both NFI and NFIII (Lanes 1-3). While addition of increasing amounts of pre-immune serum had no effect on replication (Lanes 7-9), anti-OTF-1 reduced the level of replication to that seen when only NFI is present (Lanes 4-6). Using the cloned adenovirus 4 origin, which lacks a functional NFI binding site and which contains a perfect copy of the octamer motif within the NFIII binding site, as template (26) the same inhibition was observed (not shown). We conclude that anti-OTF-1 antibodies inhibit the stimulation of adenovirus DNA replication by NFIII.

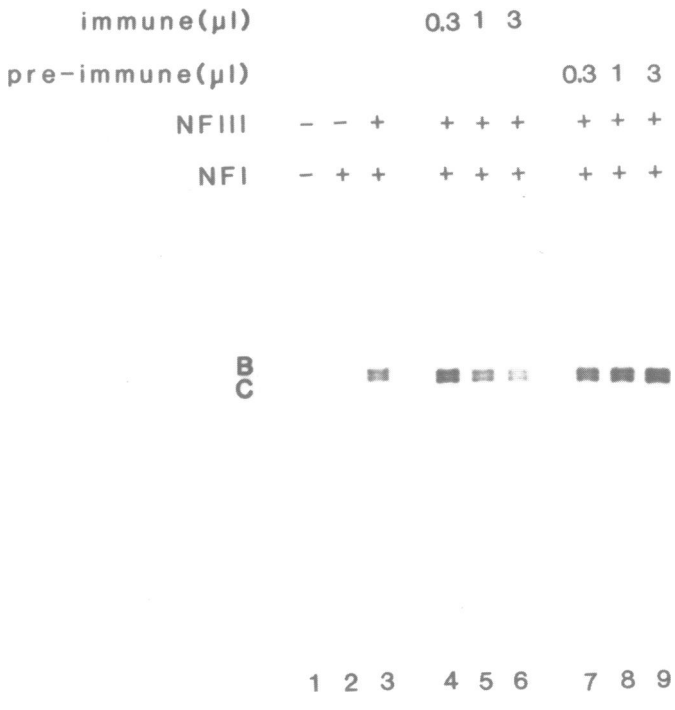

Figure 7. Inhibition of adenovirus DNA replication by anti-OTF-1. Replication of origin-containing DNA fragments of the adenovirus 5-terminal protein complex (XhoI B and C fragments) was studied in a reconstituted system as described (41). Stimulation of DNA replication by NFI and NFIII is shown in lanes 1-3. Lanes 4-6 show the effect of preincubating NFIII with increasing amounts of anti-OTF-1 antiserum. Lanes 7-9 show the effect of identical amounts of pre-immune serum.

OTF-1 stimulates Ad DNA replication

We compared the effects of OTF-1 and NFIII on adenovirus DNA replication in vitro. As templates artificially constructed adenovirus origins composed of the major origin elements were used. pEI0III contains the core origin, a high affinity NFI binding site and a high affinity NFIII binding site in an arrangement comparable to the Ad5 origin while pEI lacks the NFIII binding site. NFIII and OTF-1 were previously titrated with an Ad4 probe to determine the binding activity and the same amounts of DNA binding units were added in the replication assay and the level of stimulation was calculated by counting the radioactivity in the bands (Fig. 8). NFIII and OTF-1 stimulate

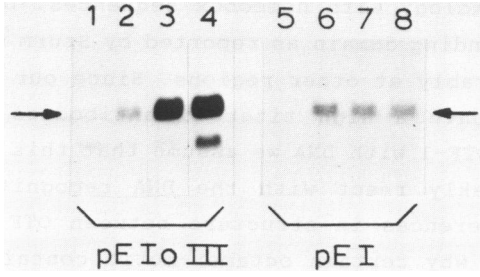

Figure 8. Stimulation of adenovirus DNA replication by OTF-1. Replication of origin-containing EcoRI-NdeI fragments of pEIOIII, containing a functional NFI and NFIII site (lanes 1-4) or pEI, lacking an NFIII site (lanes 5-8). The arrows indicate the pTP-primed, replicated DNA. Lanes 1,5 :without nuclear factors. Lanes 2,6 :including NFI. Lanes 3,7 :NFI and 14 binding units NFIII. Lanes 4,8: NFI and 14 binding units OTF-1. A binding unit is defined as the amount of protein capable of binding ,in a band shift assay, 1 fmol of a 95 bp Ad4 fragment containing the octamer sequence.

DNA replication 3,5 fold and 3,4 fold, respectively (lanes 3 and 4, compared to lane 2). No stimulation was observed with a template lacking the octamer sequence (lanes 7 and 8). We conclude that the specific activity of NFIII and OTF-1, i.e. the stimulating activity per binding unit, are comparable.

DISCUSSION

OTF-1 has been purified from HeLa cells by methods different only in minor detail from published procedures (20, 23) An antiserum raised against the purified protein has been shown to recognize both HeLa OTF-1 and a related Xenopus protein specifically, indicating that this protein, which is apparently ubiquitously expressed, is conserved between (at least) vertebrate species. The same antiserum does not however react at similar serum concentrations with human OTF-2, a lymphoid specific transcription factor which recognizes the identical DNA sequence (16, 17, 21). Since the antiserum is polyclonal this indicates that OTF-1 and OTF-2 are significantly different antigenically. Recently, several groups reported the cloning of the OTF-2 gene (48, 49, 50). Sequence comparison as well as deletion studies defined a novel DNA binding domain (POU-domain)

which shares homology with homeobox sequences. OTF-1 possesses a similar DNA binding domain as reported by Sturm et al. (51) but differs considerably at other regions. Since our antiserum does not appear to have a high titer of antibodies which prevent interaction of OTF-1 with DNA we assume that this antiserum does not or only weakly react with the DNA recognition domain of OTF-1. The differences in structure between OTF-1 and 2 might help to explain why certain octamer-motif containing promoters and enhancers can be activated in cells containing the lymphoid-specific OTF-2 but not in cells containing only OTF-1 (10, 52, 53, 54). Presumably OTF-1 and 2 interact differently with other components of the transcription machinery such that they are only capable of stimulating transcription when present in combination with a specific subset of other promoter or enhancer elements, or when these elements have a specific topology.

The data presented here, in combination with previous data on the specific interaction of purified OTF-1 and NFIII with DNA (15, 20, 26, 27, 43), demonstrate that OTF-1 and NFIII are either extremely closely related or identical. First, migration of OTF-1 and NFIII in SDS-polyacrylamide gels is indistinguishable. Second, the antiserum raised against OTF-1 recognizes NFIII. Third, stimulation of adenovirus DNA replication <u>in vitro</u> is inhibited by the anti-OTF-1 antiserum. Fourth, like NFIII OTF-1 stimulates adenovirus DNA replication. During preparation of this manuscript experiments demonstrating the physical and functional identity of OTF-1 and NFIII were also reported by O'Neill et al. (55). This is the second case in which a protein has been shown to be both a transcription factor and involved in Adenovirus DNA replication. Previously, it has been shown that CTF (CCAAT box transcription factor) and NFI were identical (56, 57). However, in contrast to the complex polypeptide composition of CTF/NFI (56, 58, 59), a single polypeptide appears to be responsible for OTF-1/NFIII activities.

Many observations indicate a close relationship between DNA replication and transcription. In E.coli, for example, a transcript hybridizing to a region near the origin can activate replication of an otherwise inert plasmid (see ref. 60). In

eukaryotic viruses like polyomavirus, SV40 and papillomaviruses, promoter sequences are located close to the origin of DNA replication (reviewed by DePamphilis, 61). In these cases activation of DNA replication could be due to the transcription process per se but in the case of adenoviruses transcription factors can activate replication in the absence of any transcription, since the stimulation is seen in an in vitro system without rNTPs and occurs at the stage where the initiation complex consisting of precursor-Terminal protein and dCMP is formed. Possibly structural changes in the DNA induced by NFIII/OTF-1 binding or clearing of non-specifically bound proteins, e.g. histones, might facilitate the assembly of multiprotein complexes functional in the initiation of both transcription and DNA replication. Adenoviruses may have adopted existing cellular transcription factors for the purpose of optimizing their replication and it will be of interest to see whether the use of transcription factors is limited to adenoviruses or will turn out to be a more general phenomenon. In this respect the presence of potential NFIII/OTF-1 binding sites in human and murine ARS elements is intriguing (62).

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