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# Adenovirus DNA-Binding Protein Forms a Multimeric Protein Complex with Double-Stranded DNA and Enhances Binding of Nuclear Factor I

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**The 72-kilodalton adenovirus DNA-binding protein (DBP) binds to single-stranded DNA as well as to RNA and double-stranded DNA and is essential for the replication of viral DNA. We investigated the binding of DBP to double-stranded DNA by gel retardation analysis. By using a 114-base-pair DNA fragment, five or six different complexes were observed by gel retardation. The mobility of these complexes is dependent on the DBP concentration, suggesting that the complexes arise by sequential binding of DBP molecules to the DNA. In contrast to binding to single-stranded DNA, the binding of DBP to double-stranded DNA appears to be noncooperative. DBP binds to linear DNA as well as to circular DNA, while linear DNA containing the adenovirus terminal protein was also recognized. No specificity for adenovirus origin sequences was observed. To study whether the binding of DBP could influence initiation of DNA replication, we analyzed the effect of DBP on the binding of nuclear factor I (NFI) and NFIII, two sequence-specific origin-recognizing proteins that enhance initiation. At subsaturating levels of NFI, DBP increases the rate of binding of NFI considerably, while no effect was seen on NFIII. This stimulation of NFI binding is specific for DBP and was not observed with another protein (NFIV), which forms a similar DNA-multimeric protein complex. In agreement with enhanced NFI binding, DBP stimulates initiation of adenovirus DNA replication in vitro especially strongly at subsaturating NFI concentrations. We explain our results by assuming that DBP forms a complex with origin DNA that promotes formation of an alternative DNA structure, thereby facilitating the binding of NFI as well as the initiation of DNA replication via NFI.**

The adenovirus DNA-binding protein (DBP) of human adenovirus serotypes 2 and 5 (Ad2 and Ad5, respectively) is a multifunctional nuclear phosphoprotein. It is essential for viral DNA replication and is also required for the control of early and late transcription. Moreover, the protein has been implicated in transformation, virus assembly, and the replication of adeno-associated viruses (for reviews, see references 2, 12, 16, and 42).

DBP is the major product of early region E2A. It is required in stoichiometric amounts during infection and accumulates to about $2 \times 10^7$ molecules per infected cell. The protein is composed of 529 amino acids (molecular weight, 59,042) (20) and has an apparent molecular weight in sodium dodecyl sulfate-containing polyacrylamide gels of 72,000. DBP is an anisometric molecule (34, 43) which can be split into two domains by mild chymotrypsin treatment (18, 39). Most of the functions ascribed to DBP are located in the C-terminal 39-kilodalton domain that also contains the nucleic acid-binding properties. The N-terminal domain, containing nearly all of the phosphorylation sites (23, 42), is required for late gene control, as indicated by mutant studies (19).

The protein was first isolated by virtue of its strong binding to single-stranded DNA (ssDNA) (44). The binding of DBP to ssDNA is weakly cooperative. At saturation, each DBP molecule covers between 9 and 13 nucleotides, and the complex has a regular, rigid, and extended configuration with a considerable tilt of the bases and a small rotation per base (41; M. E. Kuil, H. van Amerongen, P. C. van der Vliet, and R. van Grondelle, Biochemistry, in press). Binding to RNA (1, 3, 35) as well as to double-stranded DNA (dsDNA) (8, 34) has been reported. Fowlkes et al. (8) detected a specific binding to double-stranded termini by employing filter binding experiments.

We reinvestigated the binding of DBP to double-stranded origin DNA, employing gel retardation. These studies were inspired by our recent results with a cellular DNA-binding protein, termed nuclear factor IV (NFIV). Employing gel retardation, methidium propyl EDTA footprinting (13), exonuclease protection, and electron microscopy, we found that this protein recognizes DNA termini and translocates on DNA, forming a regular DNA-multimeric protein complex at saturation (4a). Since NFIV has several properties, such as binding to ssDNA, in common with DBP, we used similar techniques to investigate the binding of DBP to dsDNA. Here we show that DBP forms a multimeric protein complex with properties similar to those of NFIV. Adenovirus DNA replication occurs via a strand-displacement mechanism in which the displaced single strand is bound by DBP. The role of DBP in chain elongation also includes cooperativity with the adenovirus DNA polymerase (pol) to perform efficient displacement synthesis on double-stranded templates (22). This might be explained by a direct interaction between DBP and pol or by the existence of a specific DBP-dsDNA structure which is favorable for polymerization and strand displacement.

Although the role of DBP in elongation is reasonably well defined, its function in initiation has been less well established. The initiation of adenovirus DNA replication occurs by a protein-priming process in which the first nucleotide (dCMP) becomes covalently bound to a serine residue in the viral precursor terminal protein (pTP). This reaction requires pol, but the addition of DBP has been reported either to have no effect (32) or to stimulate initiation considerably (17, 24).

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The initiation process is enhanced 30- to 100-fold by the binding of NFI and NFIII, sequence-specific DNA-binding proteins that recognize the origin (for reviews, see references 12, 16, and 42). NFI constitutes a family of proteins probably originating from alternative splicing (14, 27, 33) and has been implicated in adenovirus DNA replication as well as in the transcriptional control of genes containing the NFI recognition sequence (14). NFIII is also very closely related or identical to a transcription factor, octamer-binding transcription factor 1 (26, 29), coded for by the *oct-1* gene (37). Both proteins are present in low amounts in HeLa cells. We show here that DBP increases specifically the binding of NFI, leading to a more efficient initiation process at low NFI concentrations.

## MATERIALS AND METHODS

**Purification of proteins.** DBP was isolated from Ad5-infected HeLa cells and purified as described previously (40), employing phosphocellulose and ssDNA cellulose chromatography. The protein concentration was determined spectrophotometrically (41). Rabbit antiserum against DBP was prepared by injection of purified DBP (100 μg per injection) into the popliteal lymph node, followed by two subcutaneous booster injections. The 39-kilodalton chymotryptic fragment was prepared as described earlier (40) and purified by ssDNA cellulose chromatography and elution with a 2 M NaCl-containing buffer. NFIV was purified to homogeneity from uninfected HeLa nuclei (4a). The purification of NFI was essentially as described elsewhere (21). The final preparation consisted of a mixture of seven bands with apparent molecular sizes between 52 and 66 kilodaltons, as estimated from sodium dodecyl sulfate-gel electrophoresis. The concentration was estimated from silver staining. NFIII was purified as described elsewhere (29).

**Gel retardation.** The electrophoretic mobility shift assay (gel retardation assay) was performed, employing a 114-base-pair (bp) fragment containing the Ad2 origin. The plasmid pHRI (11) was digested with *Eco*RI and *Xba*I, labeled at the *Xba*I site by DNA polymerase I (Klenow fragment) in the presence of [α-$^{32}$P]dCTP, and isolated by elution from a polyacrylamide gel. The specific activity of the probe was 50,000 cpm per ng of DNA. The assay mixture contained 0.2 ng of probe, DBP at the indicated concentrations, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid)-KOH (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 5 mM $MgCl_2$, 4% glycerol, 2% sucrose, 0.01% Nonidet P-40, and 0.2 μg of bovine serum albumin in a final volume of 20 μl. After incubation for 1 h at room temperature or at 0°C (for ssDNA probes), 2 μl of loading mix containing 30% sucrose was added, and the samples were loaded directly on a 4% (82:1) acrylamide-bisacrylamide gel containing 40 mM Tris, 40 mM sodium borate (pH 8.3), 1.0 mM EDTA, and 0.01% Nonidet P-40. Electrophoresis was for 3 h at 6 V/cm, and the gel was dried on Whatman DE-81 paper and exposed to film. Competition experiments were performed by mixing probe and competitor DNA before the addition of DBP.

**Footprinting.** DNase I footprinting was performed as described elsewhere, with the addition of 100 ng of poly(dI-dC) · poly(dI-dC) (30). The DNA fragment used was a 338-bp *Acc*I-*Nde*I restriction fragment from pHRI, labeled at the *Acc*I site by DNA polymerase I (Klenow fragment) with [α-$^{32}$P]dCTP. The incubation conditions were identical to those described above for gel retardation, except that 0.6 ng of DNA was used and the final volume was 50 μl. After incubation for 1 h at room temperature, the DNA was degraded with 0.15 U of DNase I at 30°C for 90 s. Further processing and gel electrophoresis were as described previously (31). The results were quantitated by densitometric scanning of the autoradiographs with a chromatogram spectrophotometer (model DU-8B; Beckman Instruments, Inc.).

**Initiation of adenovirus DNA replication.** Reaction mixtures (50 μl) contained 50 mU of highly purified vaccinia virus-expressed pTP-pol (31, 36), 0.5 μl of cytosolic extract, and 100 ng of adenovirus protein-DNA complex. The buffer was identical to the one used in DNase I footprinting, supplemented with 5 mM ATP, 1 μM dCTP, 40 μM ddATP, 5 nM [α-$^{32}$P]dCTP (3,000 Ci/mmol; Amersham Corp.), 100 μM aphidicolin, and 2.5% dimethyl sulfoxide. NFI and DBP were added at the indicated concentrations. The final NaCl concentration was 60 mM. At this salt concentration, NFI binding is enhanced by DBP to the same extent as at 100 mM NaCl.

The initiation reaction was allowed to proceed for 1 h at 37°C and stopped by the addition of sodium pyrophosphate and EDTA to final concentrations of 75 and 10 mM, respectively. Bovine serum albumin was added to 100 ng/μl, and protein was trichloroacetic acid precipitated. Proteins were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel. The incorporation of the radiolabeled nucleotide was measured by scintillation counting of gel slices.

## RESULTS

**Adenovirus DBP molecules bind sequentially to dsDNA.** We investigated the binding of purified DBP to the adenovirus origin by gel retardation. A 114-bp end-labeled fragment containing the Ad2 origin was incubated with increasing concentrations of DBP, and protein-DNA complexes were resolved on a native polyacrylamide gel (Fig. 1A). At low concentrations (10 ng/20 μl, 8.5 nM), a distinct, retarded band accompanied by a weaker band migrating slightly faster (Fig. 1A, complex 1) was observed. When the DBP concentration was increased, the complexes slowly disappeared and new complexes with more reduced mobilities were formed. These band shifts were not observed after preincubation of the DBP preparation with a highly specific antiserum against DBP, excluding the possibility that the pattern was caused by a contaminant in the purified DBP preparation (data not shown). At 60 ng, the probe was saturated. The complexes designated 1 to 4 were separated clearly in the gel, while the higher complexes were less well resolved. A weak, faster-migrating band is also observed in these higher-order complexes.

On the basis of similar results with NFIV (4a), we assume that the different complexes arise by sequential binding of DBP to the double-stranded fragment. If complex 1 contains one DBP molecule, we estimate that a total of five or six DBP molecules cover this fragment at saturation.

We compared the binding to ssDNA of similar length in this assay by incubating DBP with the same fragment after denaturation (Fig. 1B). At low DBP concentrations, two main bands are observed, one representing the free ssDNA and one migrating at the same position as a saturated ssDNA-DBP complex. We explain this result by the cooperative binding mode of DBP to ssDNA (43; Kuil et al., in press). Clearly, the binding pattern differs from that observed with dsDNA. Therefore, the bands obtained with dsDNA are not caused by the unwinding of the double-stranded fragment by DBP. In addition, at high DBP concentrations, a band appears at the position of the free

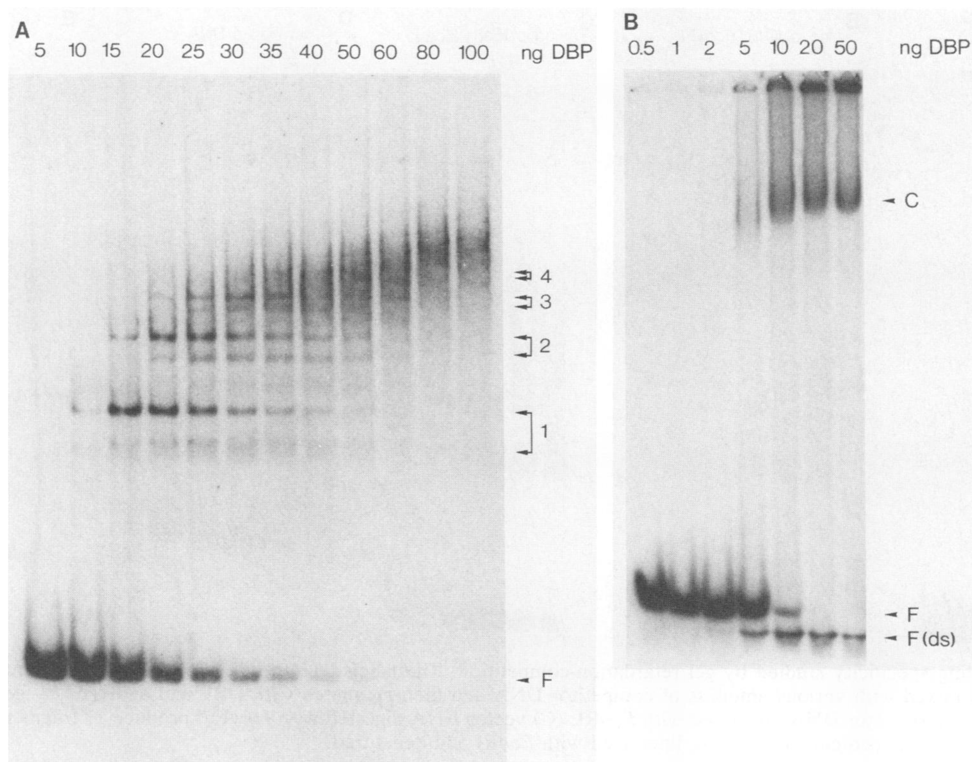



FIG. 1. Binding of DBP to dsDNA analyzed by gel retardation. (A) A 114-bp fragment containing the Ad2 origin (see Materials and Methods) was incubated with increasing amounts of DBP as indicated and electrophoresed on a native polyacrylamide gel. F, Free DNA. Protein-DNA complexes are designated 1 to 4 and indicated with arrowheads. (B) The same DNA fragment was heat denatured and incubated (on ice) with increasing amounts of DBP as indicated. F, Free ssDNA; F(ds), renatured dsDNA; C, DBP-ssDNA complex.

double-stranded probe, which might be explained by increased renaturation in the presence of DBP.

**Requirements for binding to dsDNA.** Previous analysis of NFIV showed that this protein requires molecular ends to bind to dsDNA (4a). To investigate the requirements for DBP, we performed gel retardation competition experiments. The radiolabeled 114-bp Ad2 probe described above was incubated with subsaturating amounts of DBP in the presence of increasing amounts of nonradioactive competitor DNA. Decreased binding, as shown by the appearance of faster-migrating bands, indicates affinity to the competitor DNA. Using this assay, we found that circular pUC18 DNA could bind as well as *Eco*RI-linearized DNA or plasmid DNA that was digested with *Hha*I, producing 17 fragments (Fig. 2A to C). Adenovirus DNA containing the terminal protein also competed effectively (Fig. 2D). This indicates that, unlike NFIV, DBP does not require molecular ends for binding.

We compared the affinity of ssDNA and dsDNA by competition with heat-denatured pUC18 DNA (Fig. 2E) as well as with single-stranded M13 DNA (data not shown). Both DNAs competed as effectively as the homologous dsDNA. Plasmids containing the origin competed as well as vector DNA alone did (data not shown). Thus, under these conditions, DBP binds ssDNA with the same affinity as dsDNA. Furthermore, no specificity for a particular DNA sequence was observed.

Limited chymotrypsin digestion separated DBP into a 174-amino-acid-long N-terminal fragment and a 355-amino-acid-long C-terminal fragment (40). The latter 39-kilodalton fragment is active in DNA replication and contains the ssDNA- and RNA-binding properties. After purification, we tested this fragment for its binding to dsDNA, using gel retardation. Although the efficiency of binding was slightly reduced, the fragment also bound dsDNA, producing a multimeric protein complex similar to that of the intact DBP molecule (data not shown).

**DBP increases the binding of NFI but not of NFIII.** NFI and NFIII enhance the initiation of adenovirus DNA replication by binding to their double-stranded recognition sequences in the origin. We investigated whether the complex formation between DBP and origin DNA could affect the binding of these two proteins. Since the binding of NFI and NFIII can be studied easily by DNase I footprinting, we analyzed the binding of NFI or NFIII either in the absence or presence of 1 μg of DBP by this method (Fig. 3A). Full protection of the NFI-binding site is obtained at 1.2 ng of NFI (Fig. 3A, lane 4), while in the presence of DBP, this level of protection is reached already at 0.3 ng of NFI (Fig. 3A, lane 8). Densitometric scanning of several similar experiments to quantitate the effects indicated that the binding efficiency is increased up to sixfold in the presence of DBP. No qualitative differences were observed between the footprint patterns in the presence or absence of DBP.

When we used a fixed, subsaturating NFI concentration (0.36 ng/50 μl), the amount of protection was clearly dependent on the DBP concentration (Fig. 3B, lanes 1 to 5). Even at the lowest level of DBP tested (125 ng/50 μl), the binding of NFI was increased twofold (compare 3A, lane 3, and Fig. 3B, lane 1). A plateau is reached, starting at 1 μg. As a control, we assayed the DNase I protection caused by DBP in the absence of NFI (Fig. 3B, lanes 6 to 10). No specific

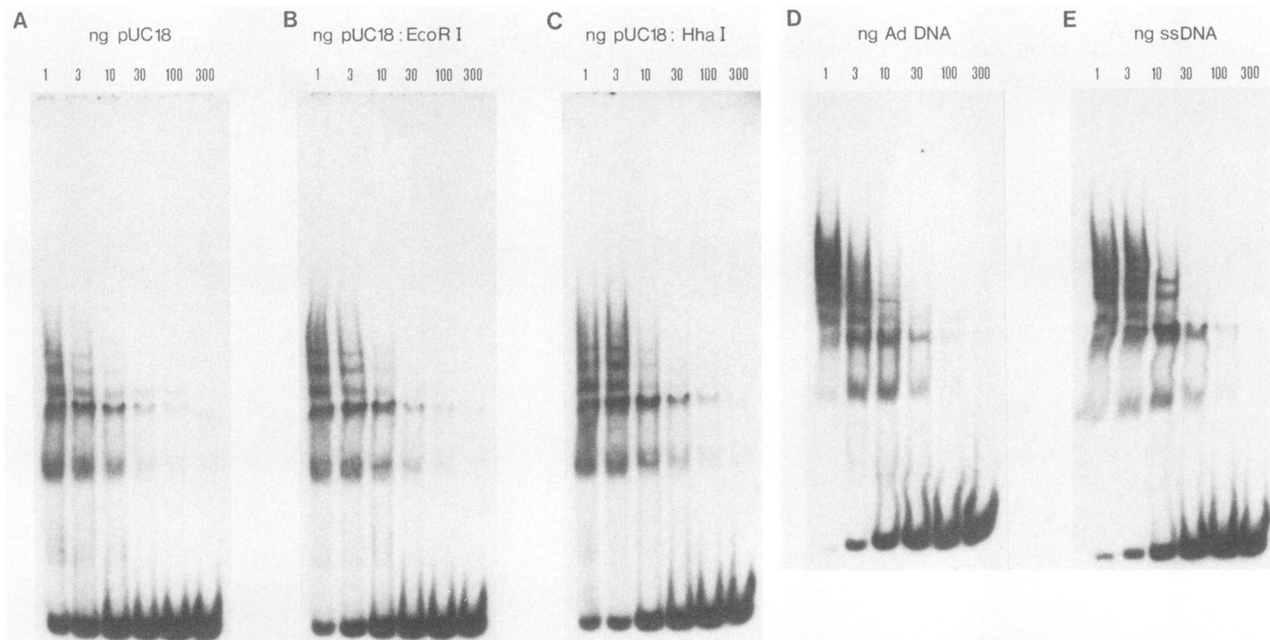



FIG. 2. DBP-binding specificity studied by gel retardation-competition. The labeled 114-bp double-stranded fragment described in the legend to Fig. 1 was mixed with various amounts of competitor DNA and then incubated with DBP and analyzed by gel retardation. (A) Vector DNA (pUC18); (B) vector DNA linearized with *Eco*RI; (C) vector DNA digested with *Hha*I to produce 17 fragments; (D) Ad2 virus DNA containing the terminal protein; (E) pUC18, linearized with *Eco*RI and denatured.

footprint was observed under these conditions. Unlike that of NFI, the binding efficiency of NFIII did not significantly change in the presence of DBP (Fig. 3C), indicating that the effect of DBP on the binding efficiency of NFI is specific and not due to a general enhancement on DNA-binding proteins. We also investigated whether increased binding of NFI was restricted to the NFI-binding site located in the adenovirus origin or could be observed with cellular NFI-binding sites. For that purpose, we employed a probe containing part of the transcriptional regulatory region of the human insulinlike growth factor II gene which contains an NFI site (4). With this fragment, the same increase in the binding of NFI by DBP was observed as that observed with the fragment containing the adenovirus origin site (data not shown).

As a control experiment, we studied the effects of NFIV on the binding of NFI. NFIV can form a DNA-multimeric protein complex with properties similar to that of DBP and thus enabled us to investigate whether formation of such a complex per se was sufficient to increase the binding of NFI. NFIV could not substitute for DBP in the reaction (Fig. 4). Even at high concentrations, no enhancement by NFIV was observed.

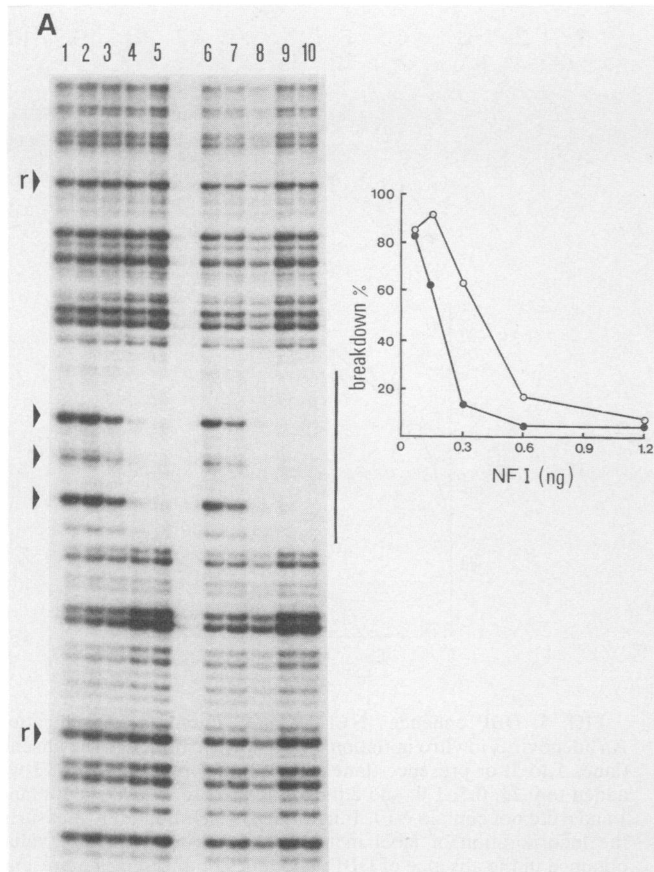
**Increased NFI binding is related to stimulation of initiation by DBP.** In order to study the functional importance of enhanced NFI binding, we measured the initiation of adenovirus DNA replication in vitro. Initiation, unlike elongation, is not critically dependent on the presence of DBP, thus enabling us to study the effect of DBP and NFI. The assay monitors the covalent coupling of the first nucleotide, a dCMP residue to the pTP.

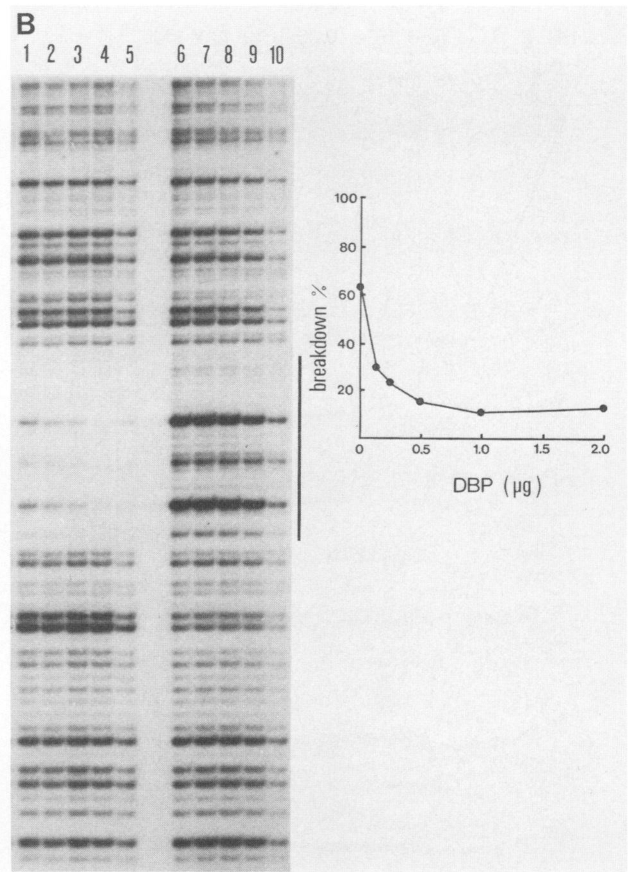
DBP stimulates initiation of replication in the absence of NFI (Fig. 5, lanes 1 and 6). Upon addition of NFI, an increase in the level of initiation is observed most clearly in the presence of DBP. A plateau is obtained at an NFI concentration which is not yet saturating in the absence of DBP. The stimulation by DBP is most pronounced at low NFI concentrations (Fig. 5, bottom) and is in agreement with the observed enhancement of NFI binding by DBP. However, since DBP also stimulates initiation in the absence of NFI, other mechanisms by which DBP exerts its stimulating effect must exist.

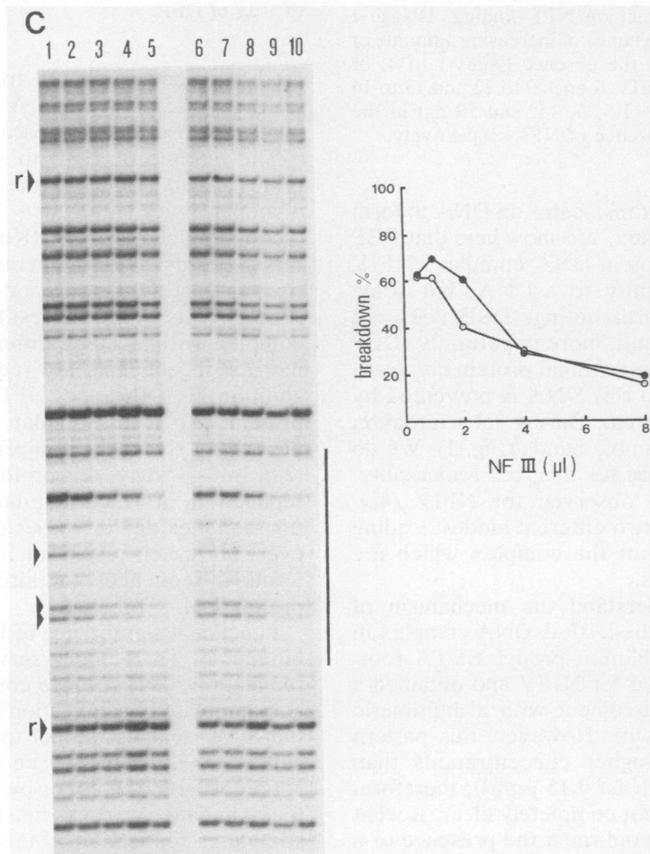
## DISCUSSION

We have investigated the binding specificity of DBP to dsDNA by employing gel retardation. One reason to study binding to dsDNA was our previous observation of a novel type of binding to dsDNA found for the HeLa NFIV, which

FIG. 3. DBP increases the binding of NFI. (A) DNase I footprints of increasing amounts of NFI (0.08, 0.15, 0.3, 0.6, and 1.2 ng) either in the absence (lanes 1 to 5) or in the presence (lanes 6 to 10) of 1 μg of DBP are shown. DNase I breakdown within the NFI-binding site (solid vertical bar next to lane 10 indicates DNase I footprint area) was quantitated via densitometric scans of the autoradiograph. Reference (r) and test bands are indicated with arrowheads. Symbols: ○, values found with NFI alone; ●, values found in the presence of 1 μg of DBP. (B) A fixed subsaturating amount (0.3 ng) of NFI (lanes 1 to 5) was incubated with increasing amounts of DBP (0.12, 0.25, 0.5, 1, and 2 μg), while as a control the same amounts of DBP were incubated without NFI (lanes 6 to 10). The breakdown was calculated and symbols are as described above for panel A. The value at 0 μg of DBP was taken from the experiment shown in panel A, lane 3. DBP alone did not decrease breakdown within the NFI-binding site. (C) Results of incubation of increasing amounts of NFIII (0.5, 1, 2, 4, and 8 μl) without DBP (lanes 1 to 5) or with 1 μg of DBP (lanes 6 to 10) are shown. The area protected against DNase I is indicated by the solid vertical bar next to lane 10. Breakdown within the NFIII site was calculated from the intensities of the indicated bands. Reference (r) and test bands are indicated with arrowheads. Symbols: ○, values obtained without DBP; ●, values obtained with 1 μg of DBP.


A
1 2 3 4 5 6 7 8 9 10
r
breakdown %
NF I (ng)


B
1 2 3 4 5 6 7 8 9 10
breakdown %
DBP (µg)


C
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NF III (µl)

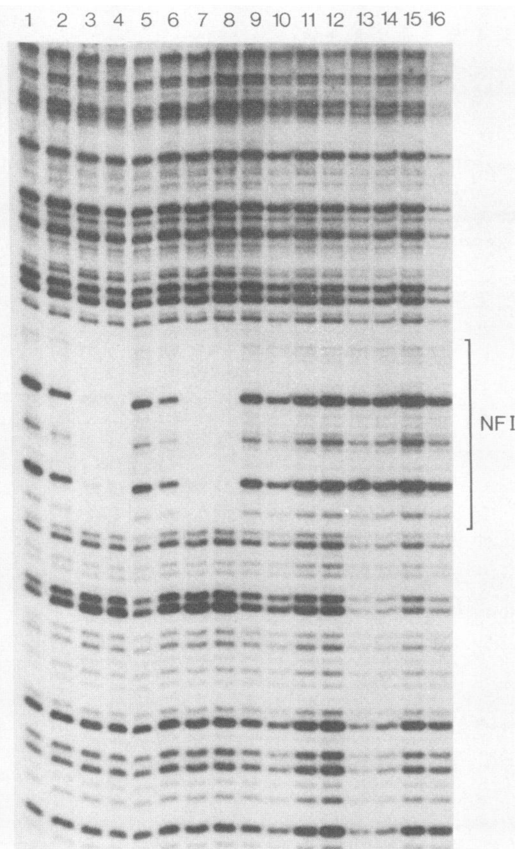


FIG. 4. HeLa NFIV has no effect on NFI binding. DNase I footprinting was performed in the presence of increasing amounts of NFI (0.15, 0.3, 0.6, and 1.2 ng) in the absence (lanes 1 to 4) or presence (lanes 5 to 8) of 5 ng of NFIV. Lanes 9 to 12 and 13 to 16 show increasing amounts of NFIV (1.5, 5, 15, and 50 ng) in the presence of 0.3 ng of NFI or the absence of NFI, respectively.

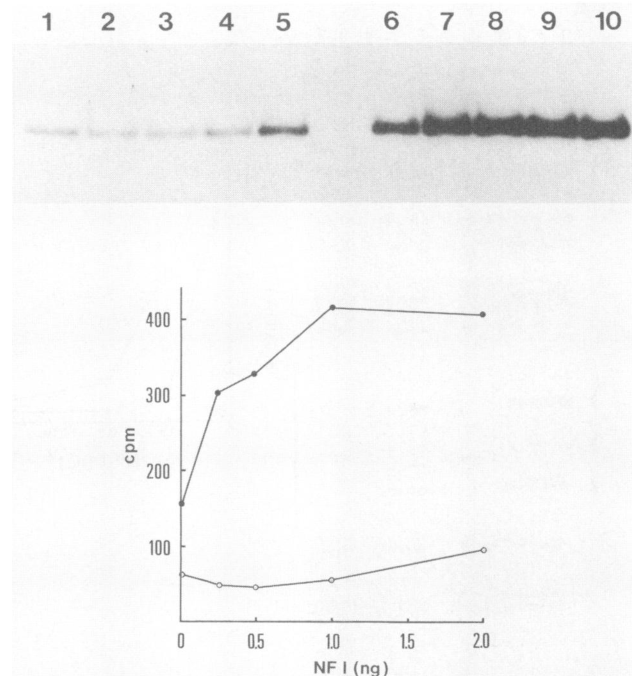


FIG. 5. DBP enhances NFI-mediated initiation of replication. An adenovirus in vitro initiation assay was performed in the absence (lanes 1 to 5) or presence (lanes 6 to 10) of 1 μg of DBP. NFI was added to 0.25, 0.5, 1.0, and 2.0 ng in lanes 2 to 5 and 7 to 10; lanes 1 and 6 did not contain NFI. Initiation was quantitated by measuring the incorporation of label in the gel slices. Symbols: ○, values obtained in the absence of DBP; ●, values obtained in the presence of 1 μg of DBP.

recognizes ends of dsDNA and translocates on DNA to form a DNA-multimeric protein complex. We show here that DBP can also form a multimeric protein-DNA complex. NFIV also shares with DBP the affinity to ssDNA, but some differences with NFIV are worth noting. DBP does not require molecular ends to bind and, more importantly, DBP recognizes the natural viral DNA-terminal protein complex, whereas the binding of NFIV to this DNA is prevented by the presence of the terminal protein. During gel retardation analysis, we always observed double bands (Fig. 1). We do not have an adequate explanation for this, but remarkably, such a phenomenon was also observed for NFIV (4a). Possibly, these proteins bind in two different modes, leading to two different conformations of the complex which are separated during electrophoresis.

At present, we do not understand the mechanism of binding or the conformation of the DBP-dsDNA complex in any detail. We performed methidium propyl EDTA footprinting similar to that performed for NFIV and obtained a regular protection pattern in agreement with a multimeric protein complex (data not shown). However, this pattern was only observed at much higher concentrations than employed in band shift (i.e., at least 0.15 μg/μl); therefore, the significance of this result is not completely clear. Recent electron microscopy data have confirmed the presence of a multimeric protein-DNA complex, while the protein-covered fragments appeared to have a bent structure (M. H. Stuiver, W. G. Bergsma, A. C. Arnberg, and P. C. van der Vliet, manuscript in preparation).

The binding of DBP to ssDNA or homopolyribonucleotides is a cooperative process (43). For binding to poly(rA), a cooperativity factor ($\omega \approx 20$ to 30) was found by circular dichroism spectroscopy (Kuil et al., in press). In contrast, binding to dsDNA fragments does not seem to be cooperative, at least on the basis of our gel retardation experiments (Fig. 1). This would suggest that the conformation of DBP in complex with dsDNA differs from that with ssDNA, but more stringent tests are required to resolve this point. In addition to DBP and NFIV, the bacteriophage Φ29 p6 protein also forms regular multimeric protein-DNA complexes, as shown by footprinting and electron microscopy (28). Interestingly, this protein is also involved in viral DNA replication, in which it stimulates the formation of an initiation complex and increases the amount of elongation. However, p6 protein does not bind to ssDNA and depends on terminal sequences to initiate formation of a multimeric protein-DNA complex.

**Functional significance of binding to dsDNA.** A priori, the binding of DBP might serve several roles in viral DNA replication. One of these could be the formation of a favorable template for elongation by the pol. It is well established that DBP enables the pol to perform efficient displacement synthesis (22); this may be caused either by a direct interaction of DBP and pol or by a change in the configuration of the template. We previously demonstrated (5) that DBP stimulates replication in the presence of NFI, while in the absence of NFI, a slight inhibition was observed. These

results were obtained in a system in which both initiation and elongation occurred, making it impossible to distinguish between these two steps in replication. It now appears that at least part of the effect can be ascribed to initiation. The function of DBP in initiation may depend on the concentration of NFI, since a significant stimulation of initiation by DBP was observed mainly under subsaturating NFI concentrations. Nevertheless, we feel that stimulation of NFI binding is not the only role of DBP in initiation, since stimulation was clearly observed even in the minimal system without NFI. Possible effects of DBP then include enhancement of the interaction between pTP-pol and the origin DNA or the providing of assistance in unwinding the origin DNA prior to initiation.

**Increase of NFI binding.** How does DBP enhance NFI binding? When studying binding as a function of time, we observed an increase in the association rate in the presence of DBP (data not shown). Preincubation of DNA with DBP gave a similar effect. This suggests strongly that NFI site occupancy is enhanced when the DNA is complexed with DBP. We cannot exclude, however, the formation of a specific NFI-DBP complex. So far, we have not detected such a complex during purification of DBP, but more rigorous tests (such as protein affinity chromatography) will be required to resolve this point.

Several other examples in which the binding of a sequence-specific DNA-binding protein is enhanced by other proteins, such as the interactions between *jun*/AP1 and the *fos* protein (45) and between herpesvirus Vmw65 and the octamer transcription factor (9, 25), have been described elsewhere. However, in these cases, direct protein-protein interactions are required, while the enhancing protein itself does not bind to DNA. This situation differs from that of NFI and DBP, which are both DNA-binding proteins. Interestingly, the HU protein, the major chromosome-associated protein in *Escherichia coli*, enhances the binding of the catabolite gene activator protein and the *lac* repressor while it inhibits binding of the *trp* repressor (7). In this case, an increase in the flexibility of the DNA by HU binding is presumed to create an easily recognizable structure. Such a situation may also apply to DBP, since differential effects are observed for NFI and NFIII. NFI and NFIII recognize the origin with different binding modes. NFI binds as a dimer at one side of the helix (6) with mainly major groove contacts, while NFIII has contacts at both sides of the helix, contacting bases in the minor as well as in the major groove (31). Their different responses to DBP might be related to this different binding mode. In this respect, it is worthwhile to note that like NFI, both catabolite gene activator protein and *lac* repressor bind predominantly at one side of the DNA helix.

Although we have no data yet, the modulating effect of DBP might not be restricted to DNA replication. Possibly, DBP also influences the binding of other factors directly involved in transcription. An indication for this is the differential effect of DBP on utilization of several early adenovirus promoters, both in vivo and in vitro (10).

Finally, one might ask whether the structure that we observe also exists in vivo. Intranuclear adenovirus DNA has an aberrant nucleosome structure and does not contain histones (15). An attractive hypothesis is that at the high DBP concentration inside nuclei, newly synthesized adenovirus DNA will be complexed with DBP rather than histones, since histone synthesis is blocked after adenovirus infection (38). If these ideas are correct, enhanced binding of NFI could then provide an efficient means to preferentially use a scarce host protein for viral replication. We do not know why the effect is restricted to NFI. Recently, a similar effect of DBP on NFI binding was observed by Cleat and Hay (2a).


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