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DNA binding properties of YB-1 and dbpA: binding to double-stranded, single-stranded, and abasic site containing DNAs

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ABSTRACT

A number of eukaryotic DNA binding proteins have been isolated by screening phage expression libraries with DNA probes containing the binding site of the DNA-binding protein. This methodology was employed here to isolate clones of the factor that interacts with the W box element of the human major histocompatibility complex HLA-DQB gene. Surprisingly, several cDNA clones of YB-1, a cDNA clone that was previously isolated with a CCAAT element-containing sequence were found. Independently, the screening of phage expression libraries with depurinated DNA resulted in the isolation of YB-1 and dbpA, a previously isolated cDNA that has homology to YB-1. Additional characterization of YB-1 showed that it bound a wide variety of DNA sequences and suggested that the binding of this protein is promiscuous. Furthermore, we show that both YB-1 and dbpA bind to depurinated DNA better than undamaged DNA and that the extent of specificity of binding is influenced by Mg^{2+}. Due to the lack of sequence specificity and high degree of binding to depurinated DNA, we suggest that these proteins might be involved in chromosome functions such as maintenance of chromatin structure or DNA repair that do not require sequence-specific binding.

INTRODUCTION

The use of labelled DNA probes to screen λgt11 expression cDNA libraries to isolate cDNAs encoding transcription factors has facilitated the rapid cloning and characterization of a number of DNA binding proteins (1–4). This procedure relies on the assumption that a phage-encoded β-galactosidase fusion protein, which is fixed to a filter, will be capable of binding to the labelled DNA in a specific manner and that no other accessory proteins are needed for correct binding. Such strategies, however, do not reveal the function of the protein beyond its ability to bind to specific or non-specific DNAs. Some of these functions may include transcription, replication, recombination, DNA repair, or maintenance/modification of chromosome structure.

The HLA-DRA and HLA-DQB genes encode the major histocompatibility complex (MHC) antigens DRαand DQβ, respectively, the expression of which is regulated in B cells by three positive regulatory elements termed the W box, X box, and Y box (reviewed in (5)). Of these, only the X box is both necessary and sufficient for B-cell expression (6). In B cells, the W box functions as a positive regulatory element (7); however, in fibroblasts it functions as a gamma-interferon response element (8,9). Specific DNA binding proteins have been shown to interact with this element (10,11). The Y box contains an inverted CCAAT box sequence. In the murine system, the Y box has been shown to bind proteins that are similar in both binding properties and primary sequence to the family of CCAAT transcription factors (CTF) proteins (12), as well as the yeast Hap2 and Hap3 proteins (13). Screening of a B-cell cDNA expression library using the Y box sequences of the HLA-DRA gene as a probe resulted in the isolation of a cDNA clone termed YB-1 (14). Interestingly, neither CTF, Hap2, nor Hap3 are similar to YB-1. As discussed below, the YB-1 and dbpA clones or their species-specific homologues have been isolated several other times. Each isolation used a different probe and sequence for the screening.

Using probes derived from the promoter sequences of the human epidermal growth factor receptor gene or the human c-erb-2 gene, Sakura *et al.* isolated two clones, dbpA and dbpB from a human placental cDNA expression library (15). Both of these clones were similar in sequence. The clone for dbpB is essentially identical to the clone isolated by Didier *et al.* (14), YB-1. Tafuri and Wolffe (16) screened a *Xenopus* oocyte λZAP cDNA expression library using a CCAAT box-containing probe and isolated the *Xenopus* homologues of YB-1 and dbpA. They further showed that the recombinant products of these clones could enhance transcription in an *in vitro* oocyte transcription system when the template DNA contained a Y box element.

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Additionally, Ozer *et al.* have isolated the rat homologue of YB-1 by using a CCAAT probe to screen a rat cDNA library (17). Yan and Tamm (18) also isolated the mouse homologue of YB-1 (99% sequence similarity) termed clone 16, during a screen for factors binding to the murine interferon response element.

In an effort to characterize the B-cell factor that interacts with the W box of another class II MHC gene, DQB, a similar approach was taken using a DQB W box probe to screen a human B-cell λgt11 expression library. Here, we report the additional isolation of a clone encoding YB-1. Independent of this isolation, was the screening of a human T-cell (Jurkat) λgt11 cDNA expression library using depurinated DNA as a probe to isolate DNA damage binding proteins that might be involved in the repair of apurinic/apyrimidinic (AP) sites. These studies, which were previously described (19), produced two clones, 3 and 11, that are now identified as dbpA and YB-1 (dbpB), respectively. The probes used to screen these libraries did not contain a CCAAT or Y box sequence. Additional data are presented that indicate that YB-1 binds to many other sequences that do not contain CCAAT box sequences. These observations suggest roles for this protein other than, or in addition to modulation of gene transcription.

MATERIALS AND METHODS

Probes

Oligonucleotides corresponding to a variety of DNA sequences were synthesized and are shown in Table I. All double-stranded probes were gel isolated prior to use and labelling. Some DNAs, including the single-stranded oligonucleotide probes, were labelled using T4 polynucleotide kinase and [γ-^{32}P]ATP. DNA that was used to screen the B-cell expression library described below was concatenated using T4 DNA ligase such that between 3 and 10 concatemers were formed. Catenated DNA was nick-translated using [α-^{32}P]dATP and DNA polymerase I. pUC18 DNA was labelled using the random primer method. These above procedures were carried out as previously described (1,3,19,20). Depurinated pUC18 DNA probes were prepared as previously described (19).

cDNA library screening

A human B-cell λgt11 cDNA library prepared from RPMI-1640 cells (Clontech, Inc., CA) was screened according to the procedure described by Vinson *et al.* (1) except that the binding buffer contained 5 mM $MgCl_2$ and did not contain EDTA. 600,000 plaques were screened using a nick-translated, catenated DQBW probe (Table I). Five independent plaques were isolated. All of these plaques represented clones of the same cDNA, differing only in their length. The screening of a λgt11 library generated from the human T-cell line Jurkat with apurinic DNA probes and the isolation of clones 3 and 11 has been previously reported (19).

Table 1

Oligo Name	Sequence*
DQBW[a]	gatcTCCAGTGCCAGGCACTGGATTCAGAACCTTCAC
DQBWL[b]	ggCAATTGAAAGACGTCCAGTGCCAGGCACTGGATTCAGAACCTTCACA
DQBY[c]	gatcCCAGTGCTGATTGGTTCCTTTCCAAGG
DQBX[d]	AAAATCTGCCCAGAGACAGATGAGGTCCTTCAGCTCCAGTG
HSV[e]	TAAAAGAAGTGAGAACGCGAAGCGTTCGCACTTCGTCCCAATATATATA
EBV[e]	gatcCAGATTAGGATAGCATATGCTACCCA

a-HLA-DQB sequences from -159 to -127 bp (33)
b-HLA-DQB sequences from -173 to -126 bp (33)
c-HLA-DQB sequences from -85 to -59 bp (33)
d- HLA-DQB sequences from -120 to -90 (33)
e- sequences from (28)
* - 5' strands of each oligonucleotide is shown; lower case letters are not part of the original sequence

DNA sequencing

For sequencing of the B-cell and T-cell derived cDNA clones, inserts were isolated following digestion with *Eco*RI and subcloned into the pBluescript vectors SK+ and KS+ (Stratagene, Inc.), respectively. Enzymatic DNA sequencing of cDNA clones was carried out using established procedures (21).

Preparation of fusion protein

Phage lysogens were created by infecting Y1089 *E. coli.* with the λ phage cDNA clones. To isolate the β-galactosidase fusion proteins lambda lysogens were induced with 10 mM isopropylthio β-galactoside and extracts were prepared as previously described (22).

Southwestern blots

NaDod-SO_4 polyacrylamide gel electrophoresis (SDS-PAGE) (23) separated fusion proteins were transferred to nitrocellulose as described (24). Filters were treated in binding buffer (25 mM Hepes-NaOH, pH 7.9, 5 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol) and 5% resuspended dried milk (Carnation, Inc.) for 20 minutes to block non-specific sites. Filters were then probed with the indicated labelled DNA (1×10^6 cpm/ml) for 2 hours at room temperature in binding buffer containing 0.25% resuspended powdered milk, then washed four times in the same buffer (5 minutes each), and exposed to X-ray film. Autoradiography was carried out overnight with or without an intensifying screen at −80°C.

Colony Southwestern blots (Plaque Binding Assays).

Plaque binding assays with depurinated and undamaged DNA probes (pUC18) were carried out as previous described (19) except that the binding solution also contained single-stranded DNA (M13mp8; 5 μg/ml) and double-stranded DNA (calf thymus; 1 μg/ml). Plaque binding assays were carried out in the presence or absence of 5 mM $MgCl_2$ with $4.5-5.0\times10^5$ cpm/ml of either depurinated or undamaged DNA.

Western blots

SDS-PAGE separated proteins were electroblotted to nitrocellulose as described (24). Filters were blocked by treatment with 5% dried milk (Carnation Inc.) at 20°C for 30 min. Filters were then stained with mouse anti-β-galactosidase antibody (Promega Inc.), in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20), for 30 min. at room temperature. Following three washes with TBST, filters were incubated for 30 minutes in TBST containing 0.2 μg/ml of a goat anti-mouse antibody conjugated to horseradish peroxidase (Amersham Inc). Following three washes, filters were developed using the BCIP/NBT detection system as described by Bethesda Research Laboratories, Inc.

Nuclear Extracts and Electrophoretic Mobility Shift Assays (EMSA)

Preparations of crude nuclear extracts from Raji cells and EMSA were carried out as described by Hasegawa *et al.* (25). DNA binding reactions contained 12 mM HEPES-KOH (pH 7.9), 12% glycerol, 75 mM KCl, 5 mM $MgCl_2$, 0.12 mM EDTA, 0.3 mM DTT, 0.5 μg of poly (dIdC)-poly (dIdC) (Pharmacia,

Inc.), 0.25 μg of sonicated salmon sperm DNA, approximately 0.5 ng of labelled DQBWL DNA, and 4 μg crude nuclear extract. Competition reactions contained the indicated amounts of either unlabelled DQBWL or DQBY DNA (Table I).

RESULTS

The W box regulatory element of the HLA-DQB gene functions as a positive regulatory element for B-cell expression (10). Miwa *et al.* showed that this region specifically bound nuclear factors contained within B-cell extracts (11). To clone the gene that encodes the DNA-binding protein(s) that interact with the W box of the DQB gene, a human B-cell cDNA expression library was screened with a radiolabelled W box containing DNA probe (Table 1). Sixty-seven clones were picked during the primary screening of approximately 600,000 recombinant phage. Of these, five were found to be positive upon subsequent plaque purification. Each of the five clones represented independent isolates of the same cDNA. The clones were designated λW13, λW19, λW47, λW49, and λW67. λW13 contained the largest insert (1.55 kb).

To evaluate further the binding activity of the recombinant clone, extracts containing the λW fusion proteins were prepared from lambda lysogens. To confirm that the λW fusion proteins were being expressed in the λ lysogens, Western blots containing protein extracts prepared from the lysogens were stained with β-galactosidase antibodies. The four λW lysogenic extracts produced a pattern consisting of three bands of higher molecular weight (Figure 1A, lanes 1–4) than those containing a random insert (lane 5), or just λgt11. To determine if these fusion proteins would bind DQB W-box DNA sequences, a Southwestern blot was carried out using a second SDS polyacrylamide gel that was run in parallel with the gel depicted in Figure 1A. This filter was probed with labelled DQB W box DNA (Figure 1B). Only the phage with the W-box binding protein inserts bound the W box DNA (lanes 1–4). These experiments suggested that the insert encoded a W box DNA-binding protein but did not exclude the possibility that it was capable of binding to other sequences as well.

DNA sequence analysis of the clone and a subsequent GENBANK homology search using the FASTA program (26) revealed virtual identity to the genes for YB-1 (14) and dbpB (15). As reported for the dbpB clone (15), homology to the dbpA gene was also found. This result was somewhat surprising and suggested that W box and Y box DNA sequences may bind the same proteins *in vivo*.

To test if B-cell extracts contained proteins that interacted with both of these sequences, an electrophoretic mobility shift assay (EMSA) was performed in which unlabelled W box and Y box containing DNAs were tested as competitors against a labelled W box DNA probe (Figure 2). Of the three bands that were resolved by the EMSA, only the upper two were competed by the autologous competitor and were thus specific for the W box containing DNA. Additional DNA competition experiments using other non-specific DNA competitors also demonstrated that these two bands were specific for W box DNA (data not shown). Only a slight competition for the Y box DNA binding proteins was detected (Figure 2) which suggested that the two sequences do not normally bind the same factor(s). Further DNA sequence analysis indicated that the two probes shared a seven basepair sequence (see Table 1). This sequence was not within the CCAAT sequence of the Y box probe, but could explain the partial competition that was observed.

In a separate effort to obtain cDNA clones that may be involved in DNA repair and recognition of damaged DNA, DNA probes, derived from the LTR region of the murine retrovirus SL3-3 (27) into which apurinic (AP) sites were introduced, were previously used to screen a T cell (Jurkat) λgt11 expression library. As described earlier, two of the cDNA clones isolated, 3 and 11, have a higher affinity for binding to depurinated DNA (AP DNA)

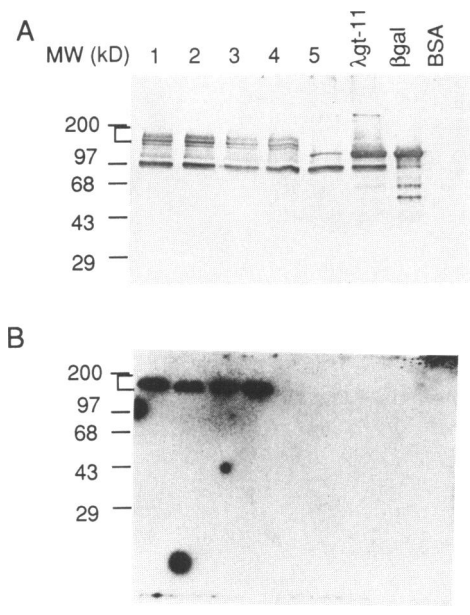

Figure 1. Western and Southwestern analysis of YB1 β-galactosidase fusion proteins. Protein extracts prepared from lambda lysogens were separated by SDS-PAGE and electroblotted to nitrocellulose filters. (A) Filters stained with β-galactosidase antisera (Fisher Inc.). (B) Filters probed with nick-translated catenated DQBW DNA (Table I). Lane: 1 λW13; 2, λW19; 3, λW47; 4, λW49; 5, random clone; λgt11 (no insert); βgal, β-galactosidase (Promega, Inc.); BSA, bovine serum albumin (Sigma, Inc.).

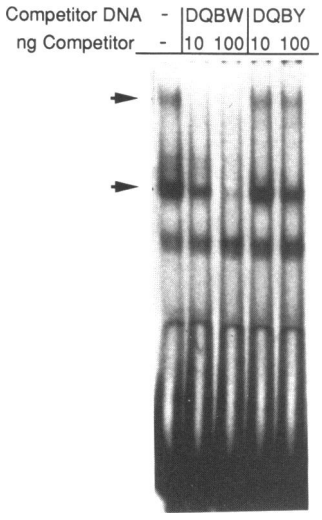

Figure 2. W box specific electrophoretic mobility shift assays. Four μg of human B-cell crude nuclear extract was incubated with 0.5 ng of labelled DQBWL DNA (W box containing DNA) as described in Materials and Methods. Competitor DNAs, either DQBW (DQBWL) or DQBY (HLA-DQB Y box containing DNA) were added at the concentrations indicated.

as compared to UV-damaged, alkylated, or undamaged DNA (19). Nucleotide sequence analysis of clones 3 and 11 showed that they were essentially identical to dbpA and dbpB, respectively (15). Thus, dbpB, YB-1, the W box-binding clones (λW), and the AP-DNA binding clone 11 are all encoded by the same gene or perhaps by a family of very closely related genes. We will refer to all of them as YB-1 from this point on.

For our YB-1 isolates (λW13 and clone 11), a single basepair difference was noted between our clone and that of Didier *et al.* Nucleotide position 485 (using the numbering of Didier *et al.* (14)) was determined to be a C; thus changing the amino acid at that position from glutamic acid to alanine. This was in agreement with the sequence of Sakura *et al.* (15). Only a partial sequence of the dbpA cDNA clone (clone 3) was determined.

Since these clones were isolated by a number of different laboratories, it appeared as though the DNA binding activity of these clones was promiscuous; binding and isolation did not depend on the presence of a particular DNA sequence. To determine if this was the case, a series of Southwestern blots using a variety of DNA sequence probes was carried out. As shown in Figure 3, extracts containing the β-galactosidase fusion protein of YB-1 were prepared from lambda lysogens and separated by SDS-PAGE. Each lane was then probed with the labelled DNA sequences indicated in Table I. DNA probes DQBWL (W-box containing DNA), DQBY (Y box containing DNA), and HSV (*Herpes simplex* virus origin of replication containing DNA (28)) exhibited strong binding to the recombinant protein. EBV (Epstein-Barr virus ori-P containing DNA (28)) and a smaller version of DQBW bound with lower affinity. Surprisingly, a single-stranded synthetic oligonucleotide of the + strand of the DQBW probe (Table 1) showed the highest affinity for the YB-1 fusion protein. The sequence of this single-stranded probe (Table 1) indicated that hairpin formation was possible, which might have resulted in some double-stranded structures. Nonetheless, the binding to this DNA is much greater than the other double-stranded species. Moreover, the binding of the DQB Y box DNA sequences to the YB-1 fusion protein was not significantly higher than that of the other DNA sequences. These results demonstrate that the YB-1 protein binds to a wide range of DNA sequences, and suggest that such binding may occur without regard to DNA sequence.

To confirm that the YB-1 (clone 11) and dbpA (clone 3) encoded proteins that bound preferentially to AP-DNA,

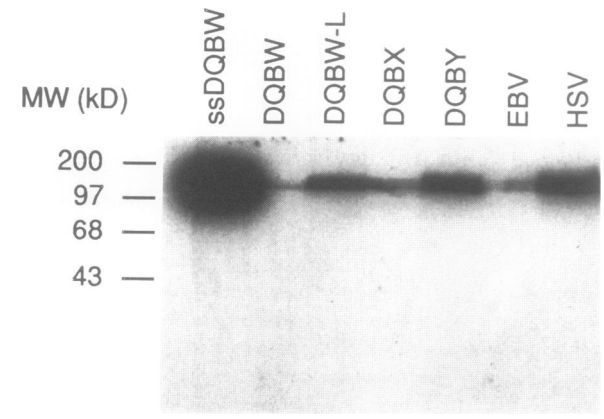

Figure 3. YB1 binds to different DNA sequences. Protein extracts prepared from YB1 expressing lambda lysogens (λW13) were separated by SDS-PAGE and blotted to nitrocellulose. Each lane was probed separately with the indicated DNA sequence (Table I). DNAs were end-labelled using T4 polynucleotide kinase as described above.

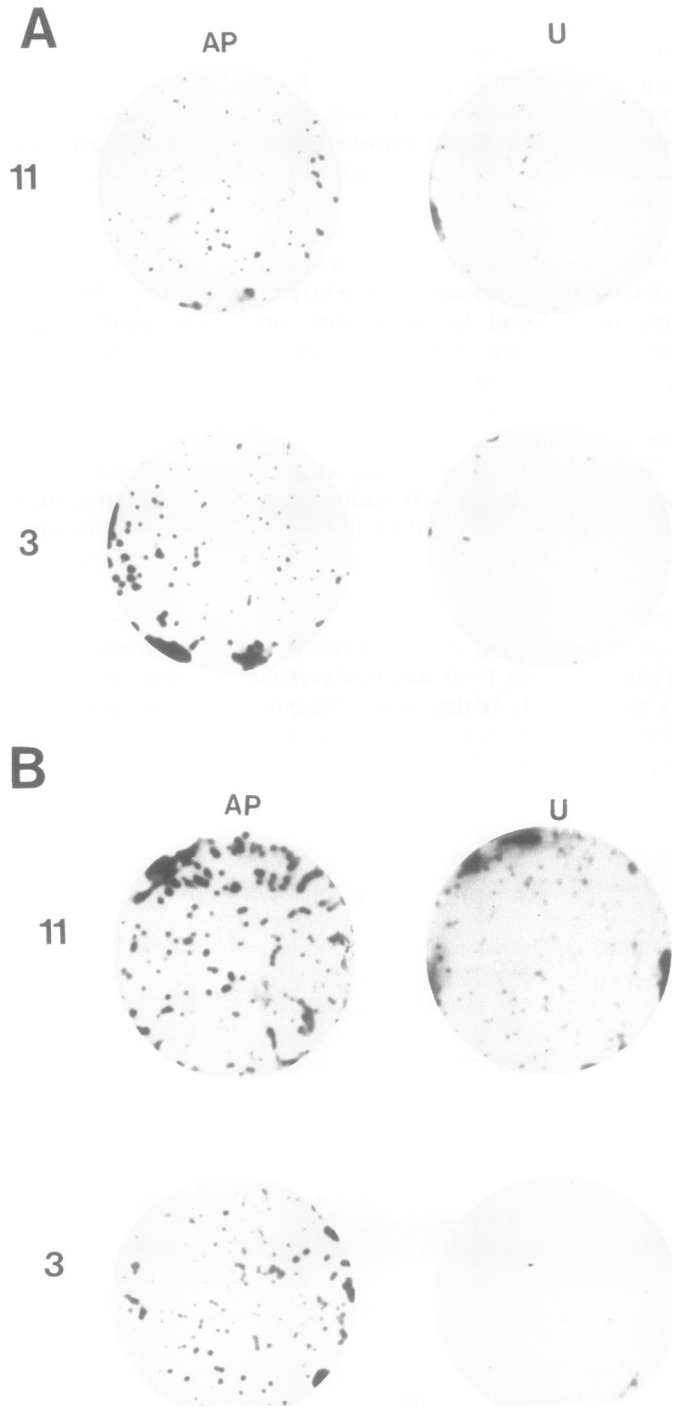

Figure 4. Binding of λgt11 recombinant clones 3 and 11 to depurinated and undamaged DNA. A.) Binding of clone 3 (dbpA) and clone 11 (YB-1) to DNA probes containing AP sites (AP) or left undamaged (U) in the presence of undamaged single stranded (5 mg/ml) and double stranded (1 mg/ml) competitor DNAs (Materials and Methods). B.) Same as A except that DNA binding was carried out in the presence of 5 mM $MgCl_2$. For the binding assays shown here, 200 plaque-forming units of cloned recombinant phage were plated in 3 ml of 0.7% agarose in LB medium.

recombinant phage containing either YB-1 or dbpA were plated and tested for their ability to bind undamaged and AP-DNA probes derived by nick-translation of pUC18 DNA. A number of damaged DNA repair enzymes have a requirement for Mg^{+2} (29), and therefore the effect of $MgCl_2$ on the ability of dbpA (clone 3) and YB-1 (clone 11) to bind to depurinated and undamaged DNA was also tested. The AP-DNA probe bound the fusion proteins to a greater extent than the undamaged DNA probe (Figure 4A). The difference in binding to depurinated versus undamaged DNA was greatest for dbpA compared to YB-1. In addition, the preferential binding to AP-DNA exhibited by both fusion proteins could be enhanced by the inclusion of 5 mM $MgCl_2$ in the binding buffer (Figure 4B). These results together with the previous studies (19) indicate that these DNA binding proteins will bind to a wide variety of DNA sequence contexts in undamaged DNA and exhibit preferential binding to depurinated DNA regardless of DNA sequence.

DISCUSSION

The cDNAs encoding the proteins YB-1 and dbpA have been cloned from cDNA expression libraries by several different groups using probes that have very limited sequence similarities. In some of the cases, the limited sequence homology included the CCAAT cis-acting element. These clones do not have similarity to clones of the CCAAT/NF1 transcription factors (CTF) that were isolated using probes designed from the amino acid sequence of purified CTF proteins (30–32). We have described two independent isolations of YB-1 and an additional isolation of dbpA utilizing DNA probes not previously employed.

We have failed to detect sequence specificity in our binding assays. In the experiments presented here, a variety of sequences from the class II MHC genes, non-MHC gene probes, and pUC18 sequences were used as substrates for the binding of YB1 and or dbpA. All of these sequences were bound by the recombinant clones with differences being only quantitative. We have also found that YB1 binds preferentially to apurinic DNA or to single-stranded-DNA. However, YB-1 does not bind preferentially to dimethylsulfate-treated or UV-irradiated DNA (19).

Several laboratories have reported that YB1 or its murine or Xenopus homologues will bind DNA with sequence specificity (14,16). How can these data be reconciled with the results presented here and by Sakura *et al.* (15), who showed binding of dbpA to four different DNA sequences? There are several possibilities. The first is that these proteins bind to a large number of sequences but not to every sequence. Second, there may be some shared DNA sequence similarities that we have not been able to identify among all of the DNAs used. Finally, preferential binding may be due to structural alterations present within the probes. For example, as shown in Figures 3 and 4 single-stranded DNA and apurinic DNA show preferential binding. It is possible that the procedures employed for labelling and processing of the DNA probes and competitors may generate unanticipated modifications, such as single-stranded or apurinic DNAs.

If the binding of these proteins is non-specific or of limited specificity, then what function can they be carrying out? A functional role for these proteins in transcription has been suggested due to the isolation of this clone in experiments designed to isolate cDNAs of transcription factors; however, direct evidence that YB1 participates in transcription has not been demonstrated. The most compelling evidence for a role of these proteins in transcription comes from the isolation and characterization of the frog homologues of YB-1, FRG Y1 and FRG Y2 (17). These proteins were shown to stimulate *Herpes simplex* virus thymidine kinase gene transcription in an *in vitro* oocyte transcription reaction. These results suggest that the *Xenopus* factors may indeed be involved in transcription in this system. It is also formally possible that FRG Y1 and Y2 may serve as general factors that enhance transcription in a non-specific manner. Still another possibility is that the *Xenopus* factors share a structural motif with the mammalian proteins yet function differently. Some insight regarding the role of these factors may also be gained from an analysis of their expression. YB-1 has been cloned from cDNA libraries from human B cells, human T cells, human placental tissue, murine fibroblasts, and *Xenopus* oocytes. Northern blot analysis ((14,15) and our unpublished results) showed that YB1 and dbpA were expressed in a wide variety of human cell lines and mouse tissues. A similar analysis with the Xenopus cDNA for FRG Y1 showed expression in a variety of tissues (16). Taken together, these observations suggest that the role of these factors is likely to be of a very general nature.

If these factors have a role in transcription then they are likely to be ubiquitous factors based on their tissue distribution and their promiscuity in binding to many different DNA sequences. On the other hand, their wide distribution, the fact that they bind to different DNA sequences, and the fact that they bind preferentially to structurally altered DNA argue that they may have some function other than, or in addition to transcription. Such functions could include a role in DNA repair, the maintenance/regulation of chromatin architecture, or participation in some other aspect of nucleic acid metabolism.

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