Volume 12 Number 10 1984 Nucleic Acids Research

The TGGCA-binding protein: a eukaryotic nuclear protein recognizing a symmetrical sequence on double-stranded linear DNA

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Received 6 March 1984; Revised and Accepted 27 April 1984

ABSTRACT

Low salt extracts of chicken oviduct nuclei contain a DNA binding protein with high affinity for specific DNA sequences in the flanking regions of the chicken lysozyme gene. Two of the three binding sites found within a total of 11 kb upstream from the promoter are located only 92 bp apart from each other. Upon comparison of the DNA binding sites, the symmetrical consensus sequence 5'-TGGCANNNTGCCA-3' can be deduced as the protein recognition site. This sequence is the central part of 23 to 25 base pairs protected by the DNA binding protein from DNAase I digestion. A homologous binding activity can be detected in nuclei from several chicken tissues and from mouse liver.

INTRODUCTION

In prokaryotes, basic genetic processes at the level of DNA depend on the recognition of DNA signal sequences by specific binding proteins (for reviews see 1-3). Evidence is emerging that specific non-histone DNA-binding proteins play the same key role for DNA dependent processes in eukaryotes.

Different classes of functional DNA elements were mapped within the transcribed and flanking sequences of eukaryotic genes and in presumed replication- and recombination-active regions of the genome. DNA sequences necessary for transcription in vitro and in vivo were found by examining the effect of specific mutations created in isolated DNA. In this way, the following controlling elements were defined: the intragenic promoters of RNA polymerase III specific genes (4-6), the initiation and TATA site of the genes transcribed by RNA polymerase II (7), several transcriptional modulator and enhancer sequences (8-11) and transcriptional termination signals (12,13).

Parallel to the discovery of controlling elements on the level of DNA, site-specific DNAase hypersensitive structures in

chromatin, which interrupt the regular nucleosomal array, could be mapped close to eukaryotic genes (14-17). The regional pattern of these chromatin sites is thought to define the state of differentiation and activity of a eukaryotic gene (18-21).

In spite of these potential sites for the interaction of proteins with DNA, only a few sequence specific eukaryotic DNA-binding proteins have been detected so far. Protein factors for which the particular DNA element plus a molecular function could be determined are the Xenopus 5S RNA gene transcription factor (4), the papova virus T-antigens (22,23) and the glucocorticoid receptor (24,25).

Using cloned linear double-stranded DNA fragments from the lysozyme gene region and low salt nuclear extracts from chicken oviduct cells in nitrocellulose filter binding assays, we have recently detected a high affinity protein DNA interaction (26). We report here details of the sequence specificity of this interaction. The twofold rotational symmetry of the protein bound sequence is typical for the type of DNA recognition as deduced for prokaryotic regulatory proteins. The discovered nuclear DNA binding protein shows no tissue specificity. A homologous binding activity could also be detected in mammalian cells.

MATERIALS AND METHODS

Preparation of nuclear protein extracts

Nuclear protein extracts were obtained according to a modified procedure described earlier (26). Fresh laying hen oviduct, liver, kidney and brain tissue and adult mouse liver tissue was homogenized with a glass teflon homogenizer in 4 volumes of homogenization buffer HB (10 mM Hepes, pH 8.0 (25°C), 0.5 spermidine, 0.15 mM spermine, 1 mM EDTA, 0.25 mM EGTA, 50 mM NaCl, 7 mM mercaptoethanol, 1 mM PMSF, 2.5 KIU/ml aprotinin and 0.5 M sucrose). All procedures were carried out at 0-4°C. The homogenate was passed through 4 layers of cheese cloth and a crude nuclear pellet was recovered by low-speed centrifugation. Three cycles of resuspension and centrifugation followed in buffer HB containing 0.5% Trition X-100. Nuclei were then washed once in 1 volume of suspension buffer SB (as HB, but 0.35 M sucrose) and once in 0.5 volume SB. If nuclei were not used directly the su-

pernatant was decanted and the pellet frozen in liquid N_2. If nuclei were processed further the nuclear pellet was resuspended in preelution buffer PB (as HB, but 10% glycerol instead of sucrose and 100 mM NaCL) and the suspension was gently shaken for 15 minutes. Recovered nuclei were then resuspended at a concentration of 1 mg DNA/ml in elution buffer EB (as PB, but 300 mM NaCL), the suspension was stirred for 30 min and centrifuged for 20 min at 12000 g. Ammonium sulfate was added to the eluate to 45% saturation and stirred for 30 min. The precipitated protein was recovered by centrifugation at 17000 g for 20 min and resuspended in 1/10 volume SB. The slightly turbid solution was clarified by centrifugation for 1 h at 50 000 rpm in a SW50.1 rotor. The supernatant, representing the DNA binding protein fraction, was divided into small aliquots and stored ab -70°C.

DNA probes for binding studies

DNA of the chicken lysozyme gene region was obtained by subcloning restricted DNA fragments from the recombinant phage λ lys 31 (27) into pBR322. Plasmid DNA and individual DNA fragments were isolated and radiolabeled as described earlier (26). Restriction enzyme digests were done according to the specifications of the commercial suppliers. For the DNA sequence analysis of the binding site regions the chemical sequencing method (28) was used on both DNA strands.

Filter binding assays

Nitrocellulose filter binding, DNA filter elution, gel electrophoretic analysis and DNA quantitation were performed as described (26) with the exception that 100 ng nonlabeled Eco R1 restricted pBR322 DNA was added as nonspecific DNA to 100 µl solution of the filter binding assay. Restriction enzyme cleavage site and nuclear protein binding site mapping using partially restricted, endlabeled DNA was done following published procedures (29).

Nuclease protection experiments

For restriction enzyme protection experiments in 100 µl assay volume 2-20 ng endlabeled DNA fragment plus 100 ng EcoR restricted pB322 were preincubated with nuclear protein extract at 75 mM NaCl (for Alu I) or 50 mM NaCl (for Hae III) in otherwise normal binding conditions for 30 min at 25°C. One to three units

restriction enzyme was added and the incubation continued for 1 to 10 min. The kinetics of restriction enzyme activity was predetermined under the same suboptimal conditions in order to adjust the incubation time for the desired partial DNA digest. Incubations were stopped by transfer into phenol-chloroform-isoamyl alcohol (25:24:1), 20 mM EDTA (pH 7.0). After deproteinization and ethanol precipitation, DNAs were analyzed on 5% polyacrylamide gels.

For DNAase I protection experiments in 100 µl assay volume 2-20 ng endlabeled DNA fragments plus 100 ng EcoR I restricted pBR322 were preincubated with 0.3 to 0.8 µg protein of nuclear extract under normal binding conditions (100 mM NaCl). After addition of 50 to 200 ng of calf thymus DNAase I the incubation was continued for one to three minutes at 25°. The kineticts of DNAase I activity was predetermined under the same conditions in order to adjust for an appropriate partial digestion pattern. Incubations were stopped and the DNA analyzed together with sequence specific chemical degradation products (28) on denaturing polyacrylamide-urea gels (30).

RESULTS

Fine mapping of protein binding sites on DNA by restriction enzyme protection

Using low salt protein extracts from chicken oviduct nuclei we have recently detected a specific protein-DNA interaction at 4 sites in the flanking regions of the chicken lysozyme gene (26). The location of DNA fragments showing preferential retention in nitrocellulose filter binding assays are shown in Figure 1a in correlation to the positions of the 4 exons of the gene. Two of these binding regions were located several kilobases upstream from the promoter of the gene. In order to determine more exactly the positions of these binding sites we used the subcloned DNA fragments between the Bam H1 site No.2 and the Hind III site No.3 (B2H3) and between the Hind III site No. 4c and the Hind III site No. 5 (H4cH5). Relevant restriction site maps of these DNA fragments are shown in Fig. 1b and Fig. 1c.

For the mapping of protein interaction sites the B2H3 596 bp fragment was labeled on either end and partially digested

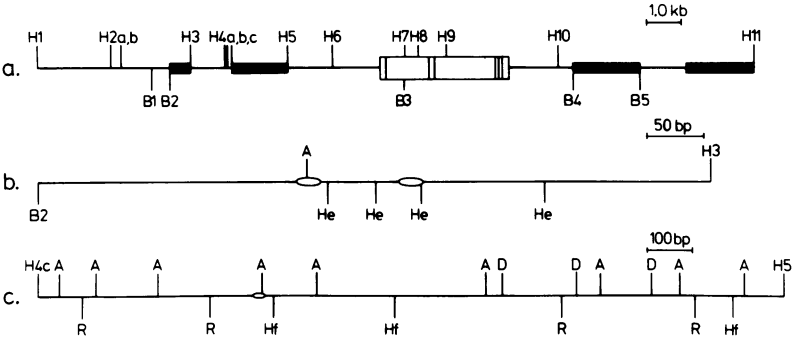

Figure 1. Map of the Binding Sites Upstream of the Chicken Lysozyme Gene. (a) Restriction map of the lysozyme gene region as contained in the recombinant phages λlys 31 and λlys 30. Cleavage sites for Hind III (H) and Bam H1 (B) are numbered in the same direction as the transcriptional polarity of the gene. The open box indicates the transcription unit and the position of the exons of the gene. DNA fragments specifically retained on nitrocellulose filters after incubation with oviduct nuclear extracts are shown as filled boxes. (b) Fragment B2H3 in enlarged scale showing cleavage sites for Alu I (A) and Hae III (He). (c) Fragment H4cH5 in enlarged scale showing cleavage sites for Alu I (A), Dde I (D), Rsa I (R) and Hinf (Hf). The positions of the protein binding sites are indicated as eyes in (b) and (c).

by Alu I or Hae III. The resulting fragments were incubated with protein extracts from oviduct nuclei and the DNA was characterized either directly or after filtration through and elution from nitrocellulose filters. As a result, it could be determined that only the larger Alu I-Hind III fragment of 358 bp and not the small Bam H1-Alu I fragment could be retained on the filter (Fig. 2, lane 1 to 4). When the fragment labeled at the Hind III site was partially digested with Hae III and analyzed in the same way, the high affinity protein-DNA interaction was found in a region to the left of the second Hae III site from the Hind III end (Fig. 2, lane 5 and 6). In summary, high affinity protein-DNA interaction must occur on the small DNA region between and including the Alu I site and the third Hae III site from the left.

Restriction enzyme protection experiments were performed in order to determine whether a binding site coincides with a restriction site. For this purpose, the alternatively enlabeled B2H3 DNA was partially digested by restriction enzymes in the presence and absence of the nuclear protein extracts, and the re-

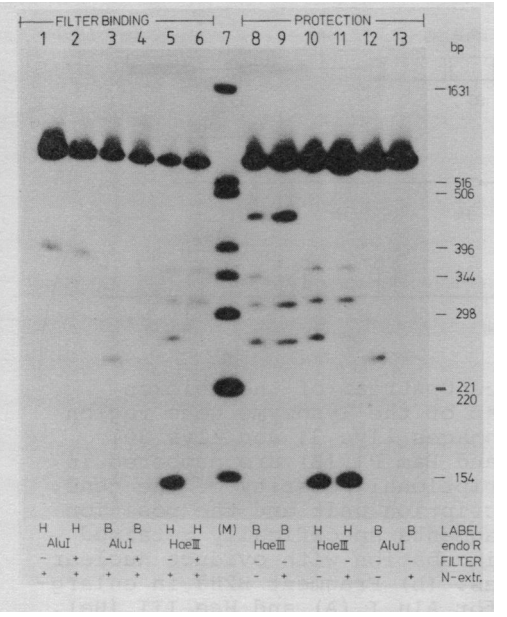

Figure 2. Mapping of BS1a and BS1b on the B2H2 Fragment by Filter Binding and Restriction Enzyme Protection Experiments. The 596 bp B2H3 fragment was 3' endlabeled at either side (B or H). For filter binding assays (slot 1-6) 1.5 ng DNA were partially digested with restriction enzymes Alu I or Hae III prior to the incubation with 1:500 diluted oviduct nuclear extract. DNA was analyzed directly (filter-) or after elution from nitrocellulose filter (filter+) on 5% polyacrylamide gels. For protection experiments (slot 8-13) the DNA was partially digested with the same restriction enzymes after preincubation with nuclear extract (+) or BSA (-) and analyzed directly. Hinf I fragments of pBR322 served as size markers.

sulting fragments were analyzed. Restriction sites protected by DNA binding proteins are expected not to be cleaved and as a result the respective bands should be missing or should be significantly reduced in intensity. As an example, when the DNA was labeled at the Bam H1 end and partially digested with Hae III in the presence of the DNA-binding protein, the 340 bp band was missing, indicating that the Hae III site 340 bp to the right of the Bam H1 site was protected (Fig. 2, lane 8 and 9). The complementary experiment is shown in Fig. 2, lanes 10 and 11. The same type of experiment with partial Alu I digestion (Fig. 2,lanes 12 and 13) indicates that the Alu I site also is protected by nuclear DNA-binding protein. Since we had already shown (ref. 26, Fig. 8) that the central 43 bp Hae III-HaeIII fragment was not retained by protein on nitrocellulose filters, we could now locate two independent binding sites (BS1a and BS1b) directly at the respective Alu I and Hae III sites on the B2H3 fragment (Fig. 1b).

For the fine mapping of the protein-binding site on the H4cH5 fragment, DNA was labeled by nick translation and digested with Alu I, Hinf I, Alu I plus Rsa I and Hinf I plus Rsa I. The

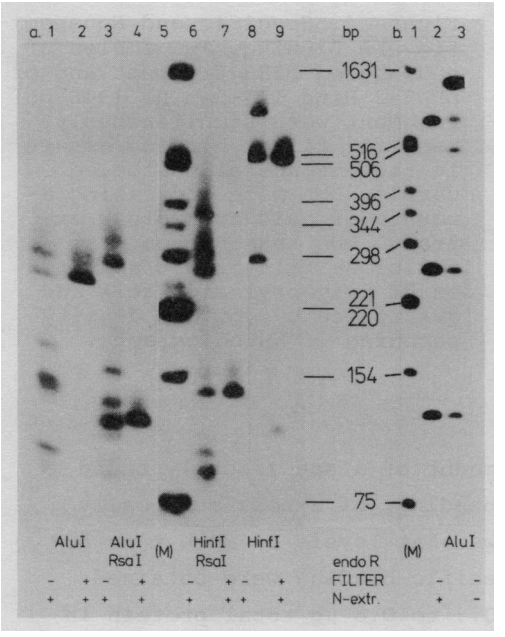

Figure 3. Mapping of BS2 on the H4cH5 Fragment by Filter Binding and Restriction Enzyme Protection Experiments. (a) The 1.65 kb H4cH5 fragment, labeled by nick translation, was used in filter binding assays (0.7 fmoles per 100 µl) after digestion with restriction enzymes (slot 1-4,6-9). (b) The 1030 bp H4c-Dde I fragment, endlabeled at the Hind III site (2 ng per) 100 µl) was partially digested with 2.5 units Alu I for 2 min after preincubation with 1:500 diluted oviduct nuclear extract (+) or BSA (-) and analyzed directly on 5% polyacrylamide gels (slot 2+3). Hinf I fragments of pBR322 served as size markers (M, slot a5 and b1).

resulting fragments were used for filter binding after incubation with the nuclear protein extract. From the pattern of retained fragments shown in Fig. 3a we can deduce that protein binding only occurs on a small Rsa I-Alu I 118 bp fragment starting approximately 380 bp downstream of the Hind III site No. H4c (Fig. 1c).

In a restriction enzyme protection experiment, the Hind III end-labeled 1030 bp H4c-Dde I fragment was partially Alu I digested in the presence and absence of nuclear binding protein. It was found that the protein-protected DNA overlaps the Alu I site 498 bp downstream of the Hind III site No. H4c (Fig. 3b and Fig. 1 c).

DNAase I protection experiments

Resolution of the nucleotide sequence covered by protein can be done with protection experiments using an unspecific DNAase i.e. calf thymus DNAase I (31). Since we were using nuclear protein extracts rather than purified DNA-binding protein, with which these experiments are usually done, optimal concentrations of the binding components were predetermined by a filter binding experiment. Varying concentrations of nuclear extract

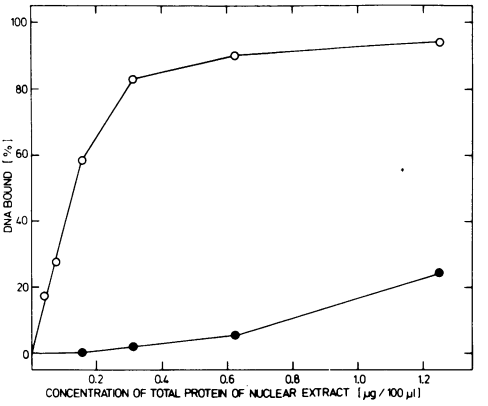

Figure 4. Specific and Nonspecific DNA Binding. Two fmoles of endlabeled B2H3 fragment and of pBR322 Hind III-Bam H1 (346 pb) fragment were simultaneously used for filter binding assays in the absence of unlabeled carrier DNA with increasing amounts of oviduct nuclear extract. DNA retained on nitrocellulose filters was analyzed on 5% polyacrylamide gels and quantified by optical density scanning of autodiographs.

were incubated with a constant amount of a specifically bound and, to account for nonspecific binding, of a nonspecifically bound DNA fragment. Figure 4 shows that levels of more than 80% specific and less than 10% nonspecific binding were obtained with 2 fmoles DNA fragments and 0.3 to 0.8 µg total protein in the nuclear extract per 100 µl assay volume. Using this narrow concentration range for the binding components, we were able to produce significantly clear protein images in DNAase I protection experiments.

Using the BS1a and BS1b carrying B2H3 596 bp fragment alternatively labeled at both ends allowed us to analyze both protein-protected DNA regions simultaneously and made it possible to distinguish protein coverage on both strands. The result of such a DNAase I protection experiment is shown in Figure 5. Two protection regions, 23 to 25 nucleotides long, can be seen on each strand. Both map at respective positions from either end and have an inner distance of 67 base pairs. The DNAase I cleavage pattern indicates a compact protein coverage with no detectable alterations of the DNAase I cleavage pattern in the surrounding DNA. The "footprint" closer to the Bam H1 site (BS1a) is significantly less pronounced, indicating a lower degree of protein occupancy at this position.

Figure 6 shows a similar analysis for BS2 on the 136 bp Rsa I-Hinf I fragment enlabeled at the Hinf I site. Here only one protected region can be detected less than 20 base pairs upstream from the Hinf I site. Again 23-25 nucleotides are protec-

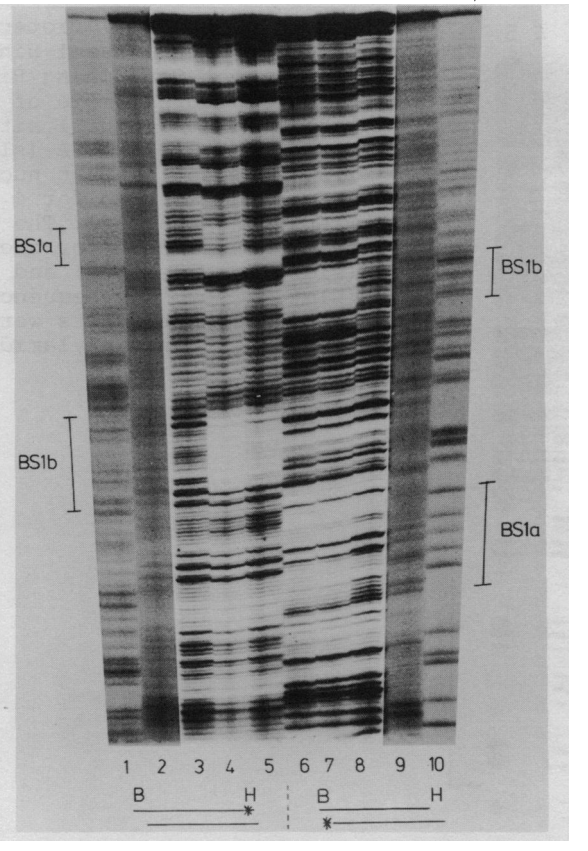

Figure 5. DNAase I Protection Experiment with B2H3 DNA (BS1a and BS1b). Two fmole fragment (slot 3-5) labeled at the Bam H1 site were preincubated with BSA (slot 3 and 8), 1:600 diluted (slot 4), 1:800 diluted (slot 5 and 6) oviduct nuclear extract and digested for 3 min with 60 ng calf thymus DNAase I. The same DNA fragments were used for chemical G (slot 1 and 10) and C (slot 2 and 9) specific sequencing reactions. DNA products were analyzed on 6% polyacrylamide-urea gels.

ted from DNAase I cleavage. Since the region which is screened in this footprint experiment (Fig. 6) covers the whole of the possible binding region, as mapped in the filter-binding experiments described in Fig. 3, we conclude that BS2 differs from the BS1 region by the fact that only a single binding site can be detected.

The consensus sequence

The same endlabeled DNA fragments which were used for

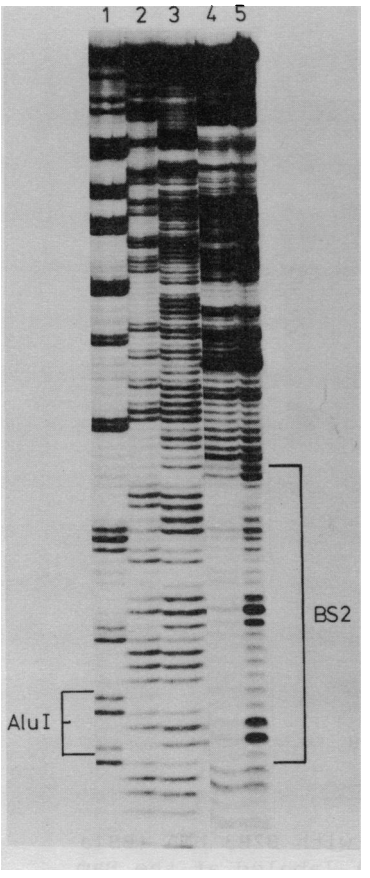

Figure 6. DNAase I Protection Experiment with a Rsa I-Hinf I 138 bp Fragment Containing Binding site BS2. Three fmole of fragment labeled at the Hinf I site were preincubated with BSA (slot 5) or 1:600 diluted oviduct nuclear extract and digested for 4 min with 80 ng DNAase I. The same fragment was used for chemical G (slot 1), C (slot 2) and C+T (slot 3) specific sequencing reactions. DNA products were analyzed on 8% polyacrylamide-urea gels.

DNAase I protection experiments were taken in parallel for chemical sequencing reactions (28). Nuclease protection profiles could therefore be mapped on the predetermined base sequences (Borgmeyer, unpublished sequences).

Two features are immediately evident on comparison of the binding sequences (Fig. 7). (1) The nuclear binding protein protects the DNA strands in a 2 to 3 base pair staggered, 3'-protruding fashion. This type of DNAase I cutting pattern is a general observation (32) and appears to reflect topological conditions for DNAase attack. (2) The central parts of the protected regions of the three binding sites BS1a, BS1b and BS2 show a partial sequence homology.

Using λ phage recombinant DNA clones in nitrocellulose

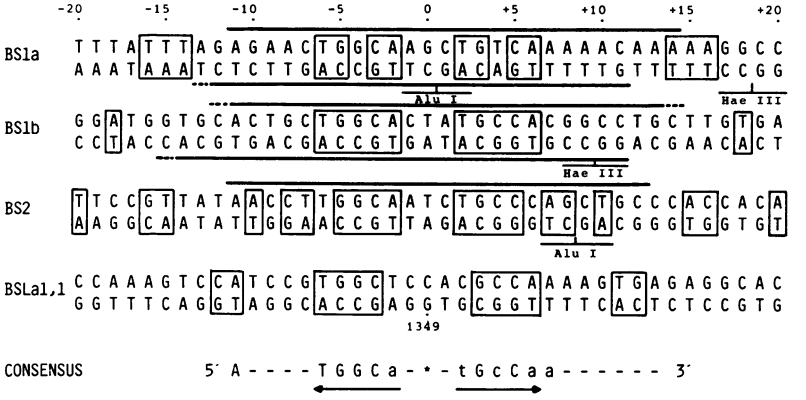

Figure 7. Sequence Comparison of Four Protein Binding Sites. The DNA sequences of the regions of BS1a, BS1b, BS2 and a binding site on the left arm of λ charon 4A (BSLa1,1) are shown, aligned according to the centers of the inverted repeats (symmetrical base pairs are boxed). The base pairs protected by the binding protein from DNAase I digestion are marked by thick lines. Positions of relevant restriction enzyme sites are indicated. The central base pair of BSLa1,1 is base pair No 1349 from the left end of λ DNA (acc. to ref. 33).

filter binding assays (26) we had previously noticed a similar high-affinity binding site on the left arm of λ charon 4A. We have mapped this binding site (data not shown) by the procedures described here to a position within the A gene (centered at position 1349, ref. 33). When we aligned all four binding sequences according to maximal sequence homology, we could deduce the consensus sequence 5'A----TGGCa-*-tGcCaa 3' (Fig.7). The consensus binding sequence contains a twofold rotational symmetry consisting of a 5 base pair inverted repeat separated by 3 freely variable central base pairs. It is conceivable that the symmetrical sequence is recognized by the binding protein. Whether the two conserved bases not included in the symmetry have any bearing on the binding process can only be decided when information of additional binding sequences is available. Since the half side of the inverted repeat is 5'-TGGCA-3' we suggest "TGGCA-binding protein" as a name for the sequence specific nuclear DNA-binding factor described here.

Occurrence of the TGGCA-binding protein in different tissues

All DNA-binding experiments described thus far were per-

formed with low salt nuclear protein extracts of laying-hen oviduct nuclei. In order to check whether the same specific DNA-binding activity is present in other cell types, we made nuclear extracts from various chicken tissues according to the same procedure used for the chicken oviduct. Comparable aliquots of protein extract on the basis of nuclear DNA content were then used in nitrocellulose filter binding assays. Nine endlabeled DNA fragments were derived from a Bam H1, Hind III double digest of a recombinant pBR322 plasmid containing the 7.0 kb Bam H1 fragment from -6.3 kb to + 0.7 kb in respect of the cap site of the lysozyme gene (B2B3; see Fig. 1a). These fragments of various sizes, including two vector fragments, were incubated with nuclear extracts from either chicken oviduct, liver, kidney or brain. As can be seen in Figure 8, after filtration through nitrocellulose the 0.59 kb B2H3 BS1-carrying fragment plus the

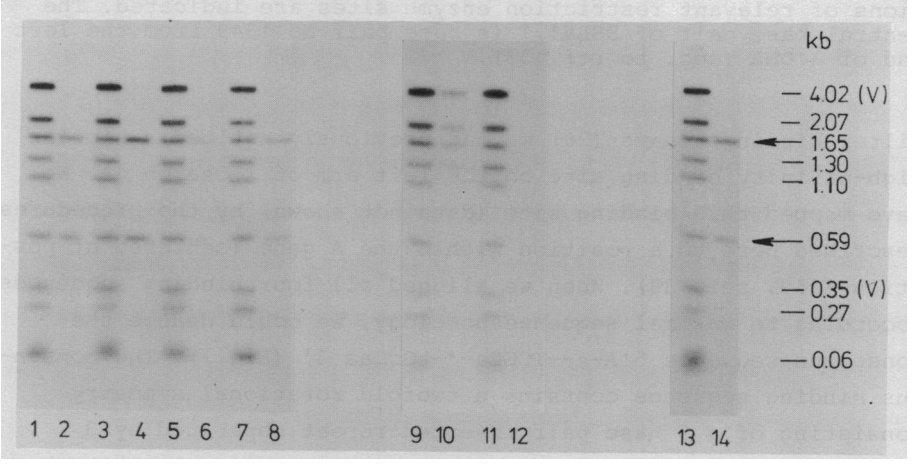

Figure 8. DNA Binding Activity of Nuclear Protein Extracts from Various Chicken and Mouse Tissues. Ten ng of endlabeled DNA fragments derived from a Hind III, Bam H1 double digest of a pBR322 recombinant plasmid containing the B2B3 7.0 Kb DNA of λlys 31 were used in filter binding assays with 1:200 diluted nuclear protein extracts from chicken oviduct (slot 1+2), liver (slot 3+4), kidney (slot 5+6) and brain (slot 7+8) and from mouse liver (slot 13+14). Control assays for nonspecific DNA binding were performed with partially purified E. coli lac repressor (slot 9+10) and for background binding with BSA (slot 11+12). Odd numbers: total incubated DNA; even numbers: filter retained DNA. Sizes of the 9 DNA fragments including two vector fragments (V) are given.

1.65 kb H4cH5 BS2-carrying fragment were retained preferently in all cases. Control incubations with BSA or E.coli lac repressor show that the preferential binding to the filter in the presence of nuclear extracts was not due to an inherent property of these two DNA fragments by themselves. The similarity of binding specificity and binding capacity in the four chicken tissues (we do not consider the differences in the amount of binding activity significant) initiated experiments to look whether the same DNA-binding activity is also present in other species. As can be seen in the right panel of Figure 8, a homologous DNA-binding activity could also be detected in mouse liver nuclei.

DISCUSSION

The characteristics of the protein-DNA interaction

In this paper we have determined the three high affinity binding sites on double-stranded DNA in the 5' flanking regions of the chicken lysozyme gene for a nuclear protein of unknown function. A stretch of 23 to 25 base pairs is protected by the binding protein on each DNA strand from digestion by DNAase I. The determination of the protein protected DNA sequence allows a number of general conclusions to be made about this protein-DNA interaction.

Competition binding experiments indicated that all sites detected in the lysozyme gene region are recognized by the same protein component (26). The sequence homology apparent in the three binding sites presents direct evidence for this conclusion. The sequence homology of the protected DNA and the fact that the central part shows twofold rotational symmetry, even though not perfect in each case, make us conclude that the sequence 5'-TGGCANNNTGCCA-3' comprises the prototype of the recognition site for the protein. Most contact points between protein and DNA must be located here. This is supported by our finding that DNA fragments to the left of the Hae III site in BS1b (ref. 26, Fig. 8) and to the left of the Alu I site of BS2 (Fig. 3) can still be bound to nitrocellulose filters with comparable strength even though the extreme right parts of the protein-protected DNA stretches are cut off. On the contrary, a cut in the homology region, as is the case with Alu I in BS1a, eliminates

detectable binding (Fig. 2, lane 4). Perfect half-sites of the recognition sequence, which occur rather frequently in tested DNA fragments, bind with an affinity below the detection level of our assay.

A protein interaction at a specific short palindromic sequence, as it is seen here, was not yet described for eukaryotic DNA-binding components. It is however likely that also a histone gene transcriptional termination factor from sea urchin (13) will have a palindromic recognition site. Twofold rotational symmetry of DNA-binding sites is common to all prokaryotic repressor/activator operator interactions (1). Such symmetrical DNA sequences allow high-affinity binding by symmetrical homomeric protein dimers on DNA double strands (34). It is likely that this principle of DNA recognition is also present in the case of the eukaryotic protein-DNA interaction described here. Several further observations support the similarity between the prokaryotic and this eukaryotic DNA recognition process. Recently, we could show in methylation protection experiments with dimethyl sulfate (35) that the eukaryotic nuclear binding protein recognizes symmetrical guanine (N7) positions in the major groove of the DNA double helix (Borgmeyer and Sippel, unpublished observation). In addition, the pattern of DNAase I cuts on DNA is unchanged in the immediate vicinity of the bound protein in our footprinting experiments (Fig. 5 and 6). A basically undistorted linear DNA structure was also found in prokaryotic repressor-DNA complexes.

Functional aspects

The data on the protein-DNA interaction do not give any direct hints on its function in the cell nucleus. If the TGGCA-binding protein exerts its function on the level of genomic DNA one would expect that "footprints" of its presence can be detected in the structure of chromatin. Using DNAase I as a probe to detect distortions from the normal nucleosomal array, we have recently mapped 8 DNAase hypersensitive sites in the chromatin of the lysozyme gene domain of the oviduct (20). One of these hypersensitive regions (HS2) includes the BS1 position. At present it is unknown to us how the TGGCA-protein can produce the local alteration of chromatin structure and whether the presence of two closely located sites BS1a and BS1b is responsible for

our finding that only BS1 maps at a hypersensitive site and not BS2. Nevertheless there is a good chance that the TGCCA-binding protein is involved in the chromatin structure of the particular hypersensitive DNA region approximately 6.0 kb upstream from the promoter of the lysozyme gene.

Expression of chicken lysozyme is regulated in the mature oviduct by all classes of steroid hormones (36). Administration of estrogens to immature chicken leads to accumulation of lysozyme mRNA in the cyptoplasm of oviduct cells (37,38). Upon hormone withdrawal transcription of the lysozyme gene is shut off and the mRNA is degraded rapidly (39). It was recently shown for two different steroid receptor proteins that they specifically bind to double-stranded DNA fragments including sequences upstream from steroid regulated promoters (24,25,40,41). Even though it is likely that steroid receptors specifically bind to the 5' flanking region of the chicken lysozyme gene (42) it is unlikely that the protein-DNA interaction described here involves steroid receptors. We think so, because we find that the TGGCA-binding protein does occur in similar amounts in cells of four different chicken tissues. Direct measurements with radioactively labeled estradiol, dexamethasone, progesterone and testosterone did not show any significant levels of steroid binding activity in our oviduct nuclear protein extracts (Nowock, unpublished results). Our additional finding that a homologous DNA-binding protein is also present in nuclei of mouse liver (see Fig. 8) and HeLa cells (unpublished results) points to a more general function for this protein.

Recently, it was described that a cellular protein factor with accessory function for adenovirus DNA replication in vitro (factor I) has double-stranded DNA binding activity (43). The protected DNA sequence in DNAase I footprinting experiments, which is part of the Adeno 5 inverted terminal repeat, includes a sequence with significant homology to the recognition sequence of the TGGCA-binding protein.Since we found that our nuclear protein extract from chicken oviduct cells does bind preferentially to the terminal 179 bp of Adeno 12 DNA in filter-binding assays (Nowock, unpublished results), there is a certain possibility that cellular factor I in Adeno-DNA replication might be identi-

cal to the binding protein described here. The level of binding protein in nuclei from chicken brain (Fig. 8) is however comparable to the level in nuclei of other tissues. This makes it unlikely that the TGGCA-binding protein is only involved in the normal cellular DNA replication process.

ACKNOWLEDGEMENT

We thank A. Steudle and R. Lange for the help with the preparation of subcloned DNA and R. Franklin and D. Dörr for help with the preparation of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 74,E5) and the Bundesministerium für Forschung und Technologie (BCT 0364/1).

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