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A liver-specific DNA-binding protein recognizes multiple nucleotide sites in regulatory regions of transthyretin, α_1-antitrypsin, albumin, and simian virus 40 genes

(tissue-specific gene expression/transcription factor/enhancer)

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ABSTRACT **Double-stranded oligodeoxynucleotides that represent protein binding sites in the regulatory regions of the mouse genes encoding transthyretin (TTR) and α_1-antitrypsin (α1-AT) bound a nuclear protein factor(s) found mainly in hepatocytes. A site in the regulatory region of the gene encoding rat serum albumin and, surprisingly, a region in the simian virus 40 enhancer also bind the same factor. Oligodeoxynucleotide affinity chromatography (with one of the TTR binding sites) allowed a 500-fold purification of the protein. The purified protein protected similar portions of all the regulatory regions, as well as the simian virus 40 core C enhancer element, from digestion with DNase I. A DNA-binding protein previously purified from liver by virtue of its ability to bind to several virus enhancer sequences also binds to TTR, α1-AT, and albumin regulatory sites. Thus, all these binding sites, which contain only minimal sequence similarity, may bind to a single protein, or a similar family of proteins, that activates liver-specific transcription of coordinately expressed genes.**

Coordinate expression of noncontiguous but related genes in *Escherichia coli* (e.g., the arginine regulon) or in yeast (galactose or amino acid loci) operates by individual negative or positive transcriptional factors (1–5). In these cases there is a single concerted purpose that unites the group of genes affected by the regulatory molecules and the term "regulon" has been used to describe such gene organization (5).

In animals, differentiated cell types such as erythrocyte precursors, muscle cells, or skin cells perform specific interrelated functions. Hepatocytes produce an amazing variety of proteins that are secreted into the serum as well as many metabolic and detoxifying enzymes not found in other tissues (6), and transcriptional control underlies most liver-specific protein synthesis (7). Should we expect the regulon concept to apply to these cases of differentiated functions in animals? Are all liver-specific proteins, regardless of their different structures and evolutionary histories, candidates for regulation by common factors? Work in this laboratory and others has been aimed at determining whether such genes are regulated by one or a few liver-specific transcription factors. As explorations of cell-specific gene control proceed, two key questions will be (*i*) are any transcription factors present in hepatocytes and not in other cell types and (*ii*) do such specific factors take part in the regulation of a group of genes expressed mainly or exclusively in hepatocytes?

MATERIALS AND METHODS

Rat Liver Nuclear Protein Extracts. Adult male Sprague–Dawley rats (300–400 g) were killed by CO_2 asphyxiation. Rat livers were dissected and rinsed, minced, and homogenized in homogenization (H) buffer (30 g/40 ml) containing 0.3% Triton X-100 at 4°C [H buffer includes 10 mM Hepes (pH 7.5), 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EGTA, 1 mM EDTA, 0.5 M sucrose, 1 mM dithiothreitol, 10 mM benzamidine hydrochloride, 0.5 mM phenylmethylsulfonyl fluoride, aprotinin at 2 μg/ml, pepstatin at 0.7 μg/ml, leupeptin at 0.7 μg/ml, and soybean trypsin inhibitor at 50 μg/ml]. The mixture was diluted with 40 ml of H buffer plus Triton X-100, filtered through cheesecloth, and centrifuged at 3000 × *g* for 10 min. Nuclei were resuspended and repelleted two more times, once with and once without Triton X-100. The final pellet was resuspended in 24 ml of H buffer and lysed with 18 ml of H buffer lacking sucrose but containing 25% (vol/vol) glycerol and 2 M KCl (final KCl concentration, 0.8 M). Nuclear proteins were extracted at 4°C with gentle agitation for 1 hr, chromatin was pelleted, and the supernatant was dialyzed for 5 hr against a 100-fold excess of 0.1 M KCl/20 mM Hepes, pH 7.5/0.1 mM EDTA/10% glycerol (two changes) and clarified by centrifugation at 10,000 × *g* for 20 min. Approximately 180 mg of nuclear protein was obtained from nuclei of 12 adult rat livers and was stored at 1–2 mg/ml at −70°C.

Column Chromatography. Heparin-agarose chromatography (5 mg of protein per ml of resin) was performed according to Briggs *et al.* (8); the gel retardation assay (9–12) was used to monitor activity. Sephacryl S-300 gel filtration, yielding a 20- to 30-kDa and a 30- to 40-kDa fraction, was performed as suggested by the manufacturer (Pharmacia Biochemicals). After application to an oligodeoxynucleotide-Sepharose column, bound protein was eluted with a 0.2–1.0 M linear KCl gradient; subsequent passages through the regenerated affinity column employed step gradients (see Fig. 2*C*) and resulted in at least a 500-fold purification (25 μg of protein).

Characterization of Binding Sites in DNA. DNase I protection assays ("footprinting") and DNA sequencing reactions were carried out as described (13, 14). Details are given in the legend of Fig. 4.*

RESULTS AND DISCUSSION

Description of Binding Sites. The protein binding sites to be considered in this work are diagramed in Fig. 1. (*i*) The binding sites of the mouse α_1-antitrypsin (α1-AT) and transthyretin (TTR) genes lie in upstream regions that act as hepatocyte (hepatoma)-specific enhancers, and the double-stranded oligonucleotides corresponding to these sites are designated TTR oligomers 2 and 3 and α1-AT oligomers A and C (9, 10, 15, 18). By competition analysis during

Abbreviations: α1-AT, α_1-antitrypsin; TTR, transthyretin; EBP20, 20-kDa enhancer-binding protein; SV40, simian virus 40; nt, nucleotide(s).

*The sequences shown in Fig. 5 are being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03829).

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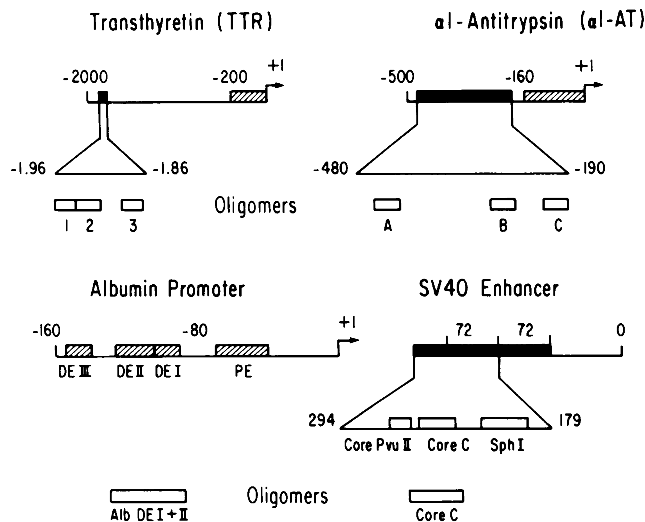

FIG. 1. Diagram of regulatory regions of genes examined in this study. Black boxes represent enhancer regions, and hatched boxes represent promoter-proximal sequences known to function in hepatocyte-specific expression. Detailed descriptions of the TTR and α1-AT sites have been given (9, 10, 15). TTR oligomers 2 and 3 and α1-AT oligomers A and C were used in the present work. The nomenclature of the albumin promoter is that of Yaniv and colleagues (16), where PE is proximal element and DE is distal element. The division of the simian virus 40 (SV40) enhancer, containing the 72-base-pair repeats, is according to Schirm et al. (17). Sequences of regions of these genes are shown in Fig. 5. Numbers indicate nucleotide (nt) positions.

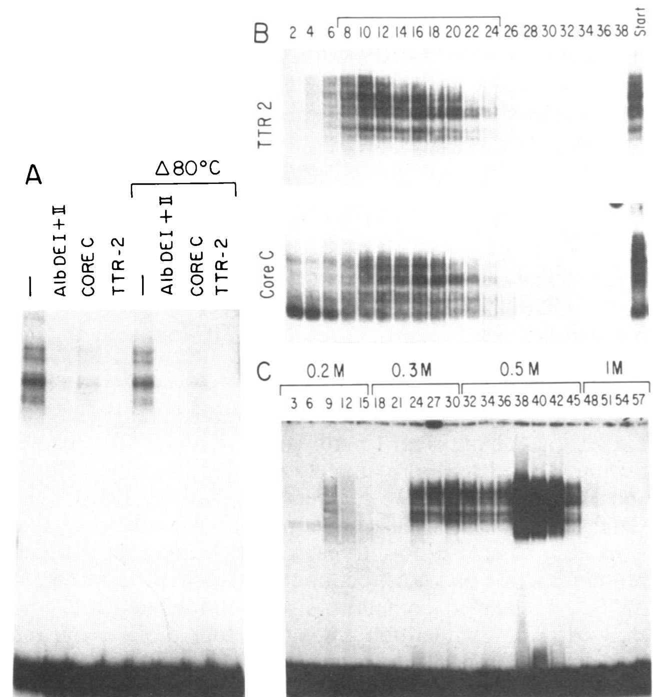

FIG. 2. (A) Gel-shift competition experiments. TTR oligomer 2 (see Fig. 1) was end-labeled and exposed to 1 μg of protein from rat liver nuclei that had been adsorbed to and eluted at 0.4–0.5 M KCl from heparin-agarose (8); electrophoresis and autoradiography were then carried out (for details see ref. 9). Competition during binding by a 20-fold molar excess of unlabeled oligonucleotide was as indicated above each lane. The four lanes on the right represent the same experiment after heating of the protein fraction to 80°C for 10 min. (B) DNA-affinity-column gradient-elution profile. After purification by chromatography on Sephacryl S-300, fractions containing proteins of 20–40 kDa and most of the TTR oligomer-binding activity were selected for affinity chromatography on a Sepharose column to which TTR oligomer 2 was coupled [40 μg/ml of Sepharose (34)]. A linear gradient of KCl (0.2–1.0 M) was used to displace bound proteins, and TTR oligomer 2 and SV40 core C oligomer were used to assay for protein binding. Binding activity located by gel shift analysis with every other column fraction is shown. (C) DNA-affinity-column step elution with KCl. The fractions indicated by brackets in B were taken for a second round of chromatography on the TTR oligomer 2 affinity column. The major fractions with binding activity for TTR oligomer 2 (fractions 24–45, eluted at 0.3 and 0.5 M KCl) were collected for a third application to the column and use in other experiments.

protein–DNA binding (gel shift) assays (11, 12), these four regions were found to interact with the same protein(s) that are at least 50-fold more abundant in liver cells than in other cells. [Both TTR oligomer 1 and α1-AT oligomer B are bound by the widely distributed transcription factor AP-1 (19, 20).] (ii) A region ≈150 nt upstream of the transcription start site of the rat albumin gene is required for low-level cell-specific expression (16, 21–25). A number of proteins are known to bind to specific sites within this 150-nt region (16, 22–24), a 45-nt portion of which (Alb DEI+II) contains two binding sites, DEI and DEII. The DEII site binds a widely distributed protein, nuclear factor I (NFI). The DEI site, with which we will be concerned, binds a protein found at a higher concentration in liver (16, 24). Finally, the SV40 promoter–enhancer region contains many different protein binding sites (26, 27). One, including the SV40 core C region (17, 28), binds the 20-kDa enhancer-binding protein (EBP20) from rat liver (29). An oligonucleotide from this SV40 region (Fig. 1, core C) has also been used in this study.

Crossreactivity in Binding Sites. We have recently demonstrated that the family of functionally important binding sites in TTR and α1-AT enhancer regions will all compete for the same factor(s) in liver extracts (9, 10, 18). We also tested a variety of other protein binding sites in DNA [e.g., those of the β-globin (30), adenovirus *E2* (31) and interferon-stimulated (32, 33) genes and the consensus site for transcription factor AP-1 (15, 19, 20)] that did not compete for binding to TTR oligomer 2 or 3 (9, 15). In one of these competition assays, we included a segment of the albumin upstream region known to be required for cell-specific expression. Since there is no significant sequence similarity between the albumin and the TTR regulatory sequences, we were surprised to find complete competition by the albumin oligonucleotide (Alb DEI + II) for protein binding to the TTR oligomer 2 (Fig. 2A). Because the SV40 enhancer was quite active during transfection in human hepatoma cells (Hep G2 cells; refs. 9, 10, and 18), we tested the SV40 core C oligomer shown in Fig. 1 and found that it competed effectively with TTR oligomer 2 for binding to protein prepared by heparin-agarose chromatography (Fig. 2A). Since McKnight and collaborators (29) had reported the purification of a heat-stable protein from rat liver nuclei that bound to the SV40, polyoma virus, and Moloney murine sarcoma virus enhancers, we heated (80°C, 10 min) the heparin-agarose protein fraction and repeated the gel-shift experiment. The heat treatment had no effect on the protein that bound to TTR oligomer 2 or on the competition by the Alb DEI + II oligomer or the SV40 core C oligomer (Fig. 2A).

We wished to determine whether a protein purified by virtue of its affinity to the TTR oligomer 2 could also recognize all of these other sequences. After heparin-agarose chromatography and size fractionation on Sephacryl-300 column, a protein fraction was purified by oligonucleotide (TTR oligomer 2) affinity chromatography (34). Fractions eluted by a salt gradient were tested by the gel-shift assay (11, 12) for binding to two labeled probes: TTR oligomer 2 and SV40 core C oligomer (Fig. 2B). The proteins in fractions 8–24 bound to both of these probes, producing very similar elution profiles for the gel-shift activity. The multiple gel-shift bands, all of which are specific for these oligonucleotides,

could be due to proteolysis of the binding factor or possibly to related but not identical binding proteins. A second distinct protein that also recognized the SV40 core C oligomer was eluted earlier from this first affinity column (bottom band in Fig. 2*B*, fractions 2–6).

A second passage through the TTR oligomer 2 affinity column was carried out with pooled fractions (Fig. 2*B*, fractions 8–24), and most of the activity for binding TTR oligomer 2 was eluted with KCl at 0.3 to 0.5 M (Fig. 2*C*). The fraction that contained gel-shift activity for TTR oligomer 2 was chromatographed a third time on the affinity column. We estimated a purification of at least 500-fold and a 50% recovery of activity during these steps.

We next used the affinity-purified protein in gel-shift assays of several different probes (diagramed in Fig. 1). SV40 core C, α1-AT oligomer C, Alb DEI + II, and TTR oligomer 2 were labeled to approximately the same specific activity and used in four separate competition reactions (Fig. 3). The same set of specific, self-competing shifted bands was found with each labeled probe. In each case, heating the purified protein fraction had no effect on the gel-shift pattern (Fig. 3, Δ80°C). Unlabeled competitors at a 40-fold molar excess showed cross-competition among the four probes, and the same quantitative results were observed with each labeled probe: competition by TTR oligomer 2 and by the Alb DEI + II oligomer was strongest, and formation of each of the complexes (gel-shift bands) was inhibited in parallel. Consistently, the SV40 core C oligomer and the α1-AT oligomer C had less ability to compete in either homologous or heterologous reactions.

These results suggested that one, or a closely related group of, liver-specific factor(s) recognized the SV40 enhancer as well as important regulatory regions of three different genes expressed in the liver. In contrast to the liver-specific control sequences (9, 10), the SV40 core C element binds nuclear proteins from a variety of cell types in which it functions (17, 27, 35, 36).

Sites of Protein Binding Assessed by DNase I Footprints. To extend the results obtained by the gel-shift experiments, DNA probes representing the α1-AT A and C sites, the TTR 2 and 3 sites, the albumin DEI site, and the SV40 core C region were assayed for regions of protein binding by DNase I footprinting (13, 37). Each of the end-labeled DNA fragments was allowed to bind samples of protein (250 and 500 pg) purified by TTR oligomer 2 affinity chromatography. A region of DNAse I protection was found in each fragment (Fig. 4). The protected regions (schematically shown by open boxes or brackets) in the α1-AT enhancer (Fig. 4 *A* and *B*) and TTR enhancer (Fig. 4*C*) included the guanine residues previously implicated by methylation-protection and methylation-interference studies as important for binding to the proteins in crude liver or hepatoma nuclear extracts (9, 10). In the α1-AT "C box," the footprint (Fig. 4*A*) was the same with 20- to 30-kDa (lanes with asterisks) or 30- to 40-kDa protein fractions (see *Materials and Methods*). The protein–DNA interactions were shown to be specific and interrelated by oligonucleotide competition during protein binding. For example, the TTR oligomer 2 blocked the DNase I footprint in the oligomer 2 and 3 regions of the TTR enhancer (Fig. 4*C*). DNase I footprints in the albumin sequence corresponding to the DEI site at base pairs −88 to −106 (16, 23, 24) was blocked by the SV40 core C oligonucleotide (Fig. 4*D*). The protected region (indicated by a bracket) in the SV40 enhancer probe (Fig. 4*E*) corresponded exactly to the core C site protected by the EBP20 protein purified by McKnight and coworkers (29) without the use of oligonucleotide affinity chromatography. Again, specificity was demonstrated by competition by the SV40 core C oligomer, which eliminated the DNase I footprint (Fig. 4*E*).

To establish whether the factor purified by TTR oligomer 2 affinity chromatsgraphy bound to the same sites as EBP20, we obtained extracts of bacteria containing a recombinant protein including 17-kDa of the EBP20 protein fused to β-galactosidase (W. Landschultz, P. F. Johnson, and S. L. McKnight, personal communication). These extracts gave the same footprint pattern as the DNA-affinity-purified protein for the oligomer 2 and 3 sites of TTR (compare Fig. 4*C* to Fig. 4*C*′).

Discussion and Sequence Comparison. We have shown that both protein purified by affinity chromatography on a TTR-enhancer oligonucleotide matrix and a fusion protein of the cloned EBP20 gene can specifically bind not only to the SV40 enhancer but also to several sequences required for hepatocyte-specific gene expression. Therefore, the protein isolated by affinity chromatography may function to activate a group of transcription units in the liver. This DNA binding occurs even though there is no large region of sequence similarity within the DNAse I footprint sites in the different regions (Fig. 5). There is only a short consensus sequence (TCNTACTC) with a match of five out of seven consensus nucleotides in these binding sites. (Fig. 5, dots indicate nucleotides that do not match the consensus). Mutation within the consensus region of the TTR oligomer 3 sequence greatly decreases protein binding (15) and enhancer activity in transfection experiments (unpublished results). However, the proposed consensus does not always occur at residues that are strongly protected against methylation with dimethyl sulfate (Fig. 5). Furthermore, no good alignment could be found between the proposed consensus sequence and the SV40 core C sequence; the best match was four of the eight consensus residues within the footprinted regions. Finally, we point out that the proposed consensus binding site of EBP20 (GTGGAAA) within the SV40 core C element (29) does not appear within the DNAse I-protected regions of any of the three liver-specific control elements presented here (Fig. 5). The SV40 core C sequence may have evolved so that it can still bind the liver-specific factor as well as related nuclear proteins present in other cell types where this enhancer can function (17, 27, 35, 36).

These results suggest that one DNA-binding protein of less than 200 amino acids may specifically recognize several

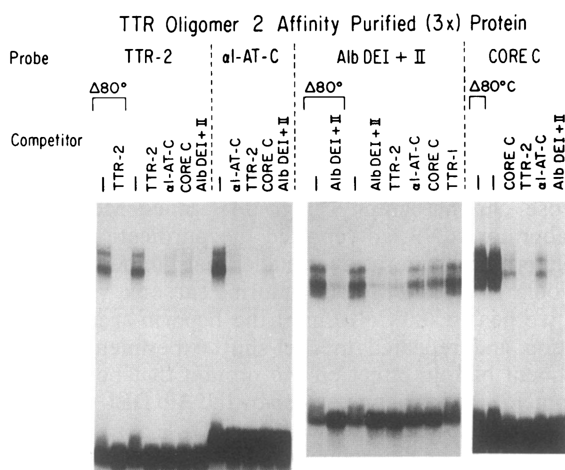

FIG. 3. Binding of affinity-purified protein to various oligodeoxynucleotides as shown by gel shift assays. The indicated double-stranded oligodeoxynucleotide probes were end-labeled to the same specific activity. Gel shift experiments (11, 12) were carried out with protein purified by three passages over a TTR oligomer column (one gradient elution and two stepwise elutions). Where indicated above the lane, a 40-fold molar excess of unlabeled oligonucleotide competitor was included in the binding-reaction mixture. TTR oligomer 1 was included as a competition control, since it contains a binding site for the non-tissue-specific transcription factor AP-1.

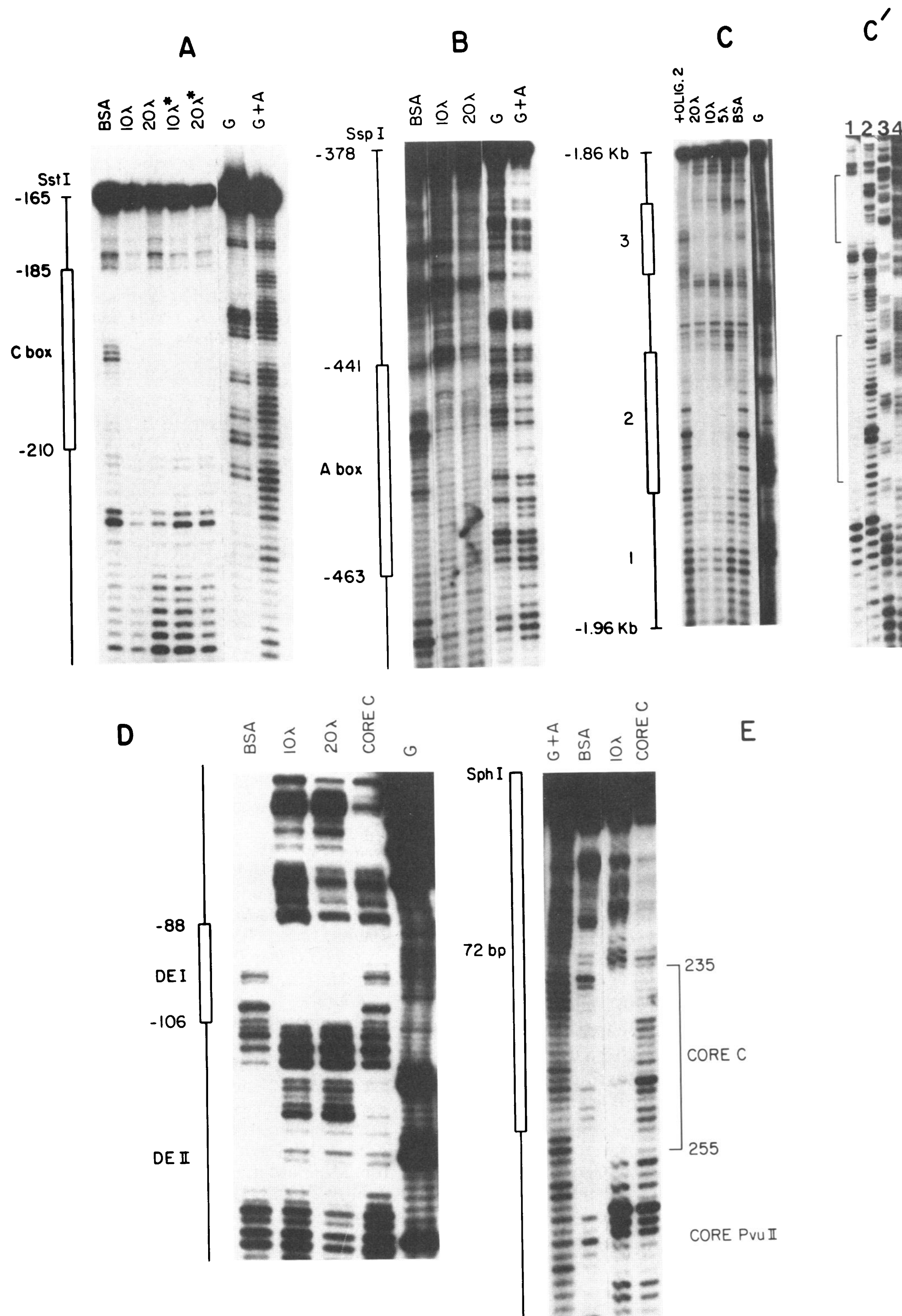

FIG. 4. DNase I footprints of various DNA samples after interaction with liver protein purified by TTR oligomer 2 affinity chromatography. End-labeled DNA containing each of the binding sites in Fig. 1 was allowed to bind affinity-purified protein [250 pg (10λ; i.e., 10 μl) or 500 pg (20λ)] and DNase I footprinting was carried out (13, 20). Products of G and G + A sequencing reactions (14) were electrophoresed in parallel to locate the protected residues. The regions protected from DNase I are indicated for each sample (boxes or brackets) along with a diagram of the probes containing various binding sites: α1-AT enhancer C- and A-box (10, 15) probes (*A* and *B*); TTR enhancer probe (*C* and *C′*) containing binding sites 1, 2, and 3 (9, 15); albumin promoter probe (*D*) containing DEI and DEII sites (16); and SV40 enhancer probe (*E*) including the core C and core *Pvu* II sites (17, 26–28). As controls, bovine serum albumin (BSA) or a 100-fold molar excess of competitor oligomer (lanes + olig. 2 and core C) were included. In *A*, two separately purified affinity fractions of approximately 20–30 kDa (lanes 10λ and 20λ) and 30–40 kDa (lanes 10λ* and 20λ*) were used. In *C′*, the TTR enhancer probe was incubated with an *E. coli* extract (gift from W. Landschulz and S. McKnight) containing an EBP20-β-galactosidase fusion protein (where the EBP20 product is estimated to be 5% of total protein) and then was digested with DNase I. Lane 2 shows the DNAse I pattern of the unbound DNA probe, and lanes 3 and 4 show the products of G and G + A sequencing reactions. bp, Base pairs; kb, kilobases.

different nucleotide binding sites. This might occur by a DNA-binding domain that could achieve several conformations or contains several overlapping DNA-recognition sites. An example of overlapping DNA-binding domains is pro-

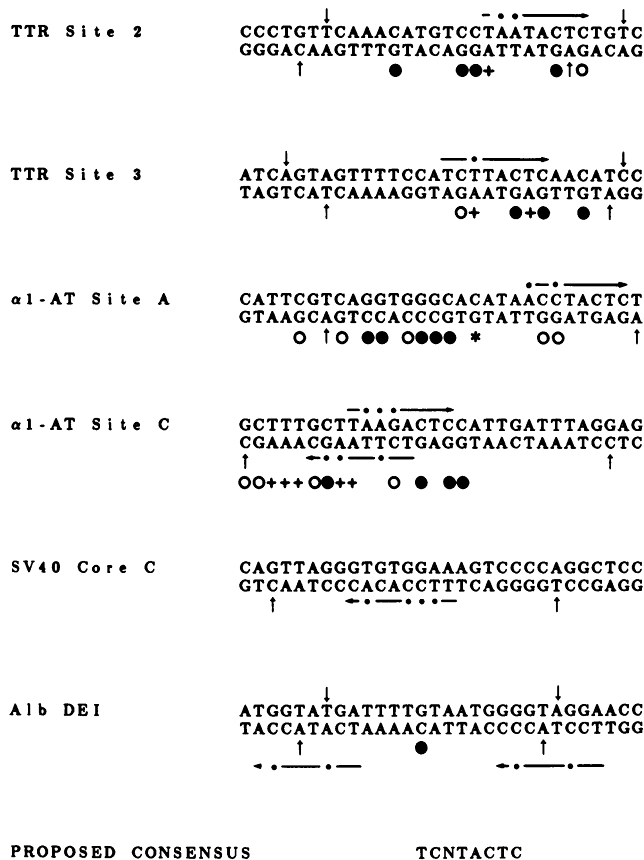

FIG. 5. Sequence comparison of regions that interact with the EBP20 protein. Positions of the regions are shown in Fig. 1. Vertical arrows indicate the boundaries of the DNase I footprint region. Other symbols are as follows: ●, guanine contact inferred from either methylation-interference or methylation-protection experiment; ○, guanine contact weakly involved; *, hypermethylated guanine residue; +, adenine residue protected. Horizontal arrow indicates position of match with consensus TCNTACTC; dots in arrow indicate nucleotides that do not match the consensus.

vided by the Hap I transcription factor in yeast, which recognizes regulatory sequences in two cytochrome *c* genes that share only 40% sequence identity (38). Alternatively, we may be unable to recognize the important similarities in DNA binding sites by simply comparing the nucleotide sequences; recognition of these similarities may require a more sophisticated comparison of such DNA structural features as bending, cruciform formation, and reactive groups in the major or minor groove. It is also possible that a family of EBP20-like proteins exists. Careful examination of a liver cDNA library and of a genomic library for related clones should help settle this issue.

Since the cDNA for the EBP20 protein has been cloned (W. Landschulz, P. F. Johnson, and S. L. McKnight, personal communication), such a search for related proteins is easily possible. In addition, by using mutant EBP20 protein and all the known available binding sites, it can be determined whether all of the obvious oligonucleotide regions described here actually bind to a single protein domain.

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1. Cunin, R., Eckhardt, T., Piette, J., Boyen, A., Pierard, A. & Glansdorff, N. (1983) *Nucleic Acids Res.* **11**, 5007–5019.
2. Johnston, S. A. & Hopper, J. E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6971–6975.
3. Hope, I. A. & Struhl, K. (1985) *Cell* **243**, 177–188.
4. Arndt, K. & Fink, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8516–8520.
5. Maas, W. K. (1961) *Cold Spring Harbor Symp. Quant. Biol.* **26**, 183–191.
6. Jungerman, K. & Katz, N. (1982) *Hepatology* **2**, 385–395.
7. Derman, E., Krauter, K., Walling, L., Weinberger, C., Raye, M. & Darnell, J. E., Jr. (1981) *Cell* **23**, 731–739.
8. Briggs, M. R., Kadonaga, J. T., Bell, S. P. & Tjian, R. (1986) *Science* **234**, 47–52.
9. Costa, R. H., Lai, E., Grayson, D. R. & Darnell, J. E., Jr. (1988) *Mol. Cell. Biol.* **8**, 81–90.
10. Grayson, D. R., Costa, R. H., Xanthopoulos, K. & Darnell, J. E., Jr. (1988) *Mol. Cell. Biol.* **8**, 1055–1066.
11. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6525.
12. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9**, 3047–3060.
13. Dynan, W. S. & Tjian, R. (1985) *Nature (London)* **316**, 774–778.
14. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
15. Grayson, D. R., Costa, R. H., Xanthopoulos, K. & Darnell, J. E., Jr. (1988) *Science* **239**, 786–788.
16. Cereghimi, S., Raymondjean, M., Garcia-Carramca, A., Herbomel, P. & Yaniv, M. (1987) *Cell* **50**, 627–638.
17. Schirm, S., Jiricny, J. & Schaffner, W. (1987) *Genes Dev.* **1**, 65–74.
18. Costa, R. H., Lai, E. & Darnell, J. E., Jr. (1986) *Mol. Cell. Biol.* **6**, 4697–4708.
19. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49**, 729–739.
20. Lee, W., Mitchell, P. & Tjian, R. (1987) *Cell* **49**, 741–752.
21. Ott, M.-O., Sperling, L., Herbomel, P., Yaniv, M. & Weiss, M. C. (1984) *EMBO J.* **3**, 2505–2510.
22. Babiss, L. E., Herbst, R. S., Bennett, A. L. & Darnell, J. E. Jr. (1987) *Genes Dev.* **1**, 256–267.
23. Gorski, K., Carneiro, M. & Schibler, U. (1986) *Cell* **47**, 767–776.
24. Lichtsteiner, S., Wuarin, J. & Schibler, U. (1987) *Cell* **51**, 963–973.
25. Heard, J.-M., Herbomel, P., Ott, M.-O., Mottura-Rollier, A., Weiss, M. & Yaniv, M. (1987) *Mol. Cell. Biol.* **7**, 2425–2434.
26. Zenke, M., Grundstrom, Matthes, H., Wintzereith, M., Schatz, C., Wildeman, A. & Chambon, P. (1986) *EMBO J.* **5**, 387–397.
27. Xiao, J. H., Davidson, I., Ferrandon, D., Rosales, R., Vigneron, M., Macchi, Ruffenach, F. & Chambon, P. (1987) *EMBO J.* **6**, 3005–3013.
28. Weiher, H., Konig, M. & Gruss, P. (1983) *Science* **219**, 626–631.
29. Johnson, P. F., Landschulz, W., Graves, B. J. & McKnight, S. L. (1987) *Genes Dev.* **1**, 133–146.
30. Myers, R. M., Tilly, K. & Maniatis, T. (1986) *Science* **232**, 613–618.
31. Kovesdi, I., Reichel, R. & Nevins, J. R. (1986) *Cell* **45**, 219–228.
32. Levy, D., Larner, A., Chaudhuri, A., Babiss, L. E. & Darnell, J. E., Jr. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8929–8933.
33. Reich, N., Evans, B., Levy, D., Fahey, D., Knight, E., Jr., & Darnell, J. E., Jr. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6394–6398.
34. Kadonaga, J. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5889–5893.
35. Mitchell, P. J., Wang, C. & Tjian, R. (1987) *Cell* **50**, 847–861.
36. Xiao, J., Davidson, I., Macchi, M., Rosales, R., Vigneron, M., Staub, A. & Chambon, P. (1987) *Genes Dev.* **1**, 794–807.
37. Galas, D. & Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3170.
38. Pfeiger, K., Prezant, T. & Guarente, L. (1987) *Cell* **49**, 19–27.