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Different liver nuclear proteins bind to similar DNA sequences in the 5′ flanking regions of three hepatic genes

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ABSTRACT

The proximal promoter region of the human transferrin gene contains an hepatocyte-specific *cis*-element (PRI, nucleotides -76 to -51) whose DNA sequence is homologous to a sequence (nucleotides -89 to -68) present in the transcriptionally essential 5' region of the human antithrombin III gene and to another hepatocyte-specific sequence (A domain) of the human α1-antitrypsin gene promoter. The results reported here lead to the conclusion that the liver *trans*-acting factor Tf-LF1, binding to the transferrin PRI *cis*-element interacts with the homologous antithrombin III region, but is different from the transcription factor LF-A1 interacting with the A domain of the α1-antitrypsin promoter.

The distal region DRI (nucleotides -480 to -454) of the human transferrin gene promoter presents in its core the same 10 nucleotide-long sequence as the PRI *cis*-element. We have previously shown that the liver protein Tf-LF2, binding to the DRI element is different from the Tf-LF1 *trans*-acting factor. In this paper we also show that Tf-LF2 is different from the transcription factor LF-A1 interacting with the α1-antitrypsin promoter.

The results allow us to conclude that at least three distinct liver nuclear proteins bind to different subsets of 5' DNA regions containing similar sequences. These sequences are present in genes expressed essentially in liver.

INTRODUCTION

Recent evidence has established that to a large extent, the transcriptional control of eukaryotic genes, tissue-specific or not, is the result of the interaction between specific DNA sequences named *cis*-acting elements and *trans*-acting proteins (1). Liver cells expressing plasma proteins constitute an attractive system to study tissue-specificity and indeed some hepatocyte-specific DNA elements have been identified (2, 3, 4). Transferrin (Tf) is an essential plasma protein mainly synthesized in the liver. We have recently demonstrated that the 5' region situated between nucleotides -119 and -45 is functionally essential for human Tf gene expression (5). Within this region we have identified an essential hepatocyte promoter element located between nucleotides -76 and -51 (Schaeffer *et al.*, submitted) named Tf-PRI (5). The core of this element is homologous to that of a transferrin distal promoter positive regulatory element, named Tf-DRI

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(nucleotides -480 to -454). It is also homologous to DNA sequences present in the 5' flanking regions of two other hepatic genes, the A domain (Aα1-AT) of the human α1-antitrypsin (α1-AT) promoter and the region situated between nucleotides -89 to -68 of the human antithrombin III (AT III) gene. This AT III DNA region is contained in a 328 bp fragment that allows gene transcription in hepatoma cells when assayed by transient expression experiments (6 and our unpublished data). In this paper we show that the *trans*-acting factor binding to the Tf PRI *cis*-element appears to be identical to the hepatic factor interacting with the AT III -89/-68 region. In addition, we demonstrate that two other liver factors interact respectively with the A domain of the α1-AT promoter and with the DRI element of the Tf gene.

MATERIALS and METHODS

Enzymes and reagents

T4 polynucleotide kinase, DNA polymerase I (Klenow fragment) and restriction endonucleases were purchased from New England Biolabs; ^{32}P-labelled dNTPs from Amersham and DMS from Aldrich Europe. All other reagents used were analytical grade or purer.

Preparation of nuclear protein extracts

Liver nuclei and nuclear protein extracts were obtained as previously described (5).

DNAse I footprints

DNAse I footprints were performed as described by Galas and Schmitz (7), with some modifications (5). Probes used in footprints were restriction fragments of the 5' region of the Tf or the AT III genes subcloned in pUC19 to facilitate their 3' end labelling. The following fragments were used: *Hin*dIII-*Pst*I (-615 to -405) of the 5' region of the Tf gene (8) and *Dra*I-*Sau*3aI (-151 to +24) of the 5' region of the AT III gene (6).

DNA-protein mobility shift assay

The standard assay (9) was performed in a final volume of 12 µl containing 1 ng of 5' ^{32}P-labelled double strand oligonucleotide (5000 cpm), 1.5 µg poly (dI-dC), 25 ng of sonicated salmon sperm DNA, 30 ng of sonicated *E.coli* DNA, 4 mM $MgCl_2$, 4 mM spermidine, 30 mM KCl, 0.25 mM EDTA, 0.5 mM DTT, 0.25 mM PMSF, 10 mM HEPES pH 7.9, 10% glycerol and 6 µg rat liver nuclear proteins. After 15 min at 4°C, the mixture was loaded onto a 6% polyacrylamide gel (acrylamide: bisacrylamide; 30:1) in 0.25xTBE (1x = 89 mM Tris, 89 mM boric acid, 2 mM EDTA pH 8.45), and electrophoresed for 90-120 min at 10 V/cm. The gel was dried and autoradiographed. For the competition experiments, various amounts of

Table I. Sequence of synthetic oligonucleotides used in this paper.

	-79 -49
Tf PRI	ACAAACACGGGAGGTCAAAGATTGCGCCCAG
	TGTGCCCTCCAGTTTCTAACGCGGGTCTGTT
	-480 -458
Tf DRI	AGTCTGTCTTTGACCTTGAGCCC
	ACAGAAACTGGAACTCGGGTCAG
	-92 -68
AT III-H	GGTCATCAGCCTTTGACCTCAGTTC
	TAGTCGGAAACTGGAGTCAAGGGCC
	-128 -98
Aα_1-AT	CCCAGCCAGTGGACTTAGCCCCTGTTTGCTC
	GGGTCGGTCACCTGAATCGGGGACAAACGAG

Numbers indicate the position of the nucleotide relative to the cap site of each gene.

unlabelled competitor oligonucleotides (Table I) were added in the binding reaction.

Heat inactivation experiments

Nuclear protein extracts (10 to 30 µg/µl) were heated 5 min at 65°C and cooled for 10 min at 4°C. The precipitate was eliminated by centrifugation.

Methylation interference assays

Probes for methylation interference assays were synthetic oligonucleotides. Each oligonucleotide was 5' end labelled, annealed with the non labelled complementary oligonucleotide and partially methylated (10). 50 µg of liver nuclear extracts were incubated with 20 ng of each probe in the presence of 20 µg of poly (dI-dC) under the conditions indicated for the DNA-protein mobility assay. After incubation at 4°C for 15 min, the incubation mixture was loaded onto a 6% polyacrylamide gel and electrophoresed as described above. Free and bound DNA were excised from the gel, eluted and ethanol precipitated (10). The obtained DNA was treated with 1 M piperidine for 30 min at 90°C, lyophilized and electrophoresed on 20% acrylamide 8 M urea gels.

RESULTS

In the human Tf 5' flanking region, proximal region PRI (nucleotides -76/-51) and distal region DRI (nucleotides -480/-454) are binding sites for liver

Table II. Comparison of the relevant DNA sequences of Tf PRI, Tf DRI and AT III-H regions.

	Position	Sequence
Tf PRI (5)	-51 / -76	GGGCGCAA<u>TCTTTGACCT</u>CCCGTGTT
Tf DRI (5)	-480 / -454	AGTCTG<u>TCTTTGACCT</u>TGAGCCCAGCT
AT III-H (6)	-89 / -68	CATCAG<u>CCTTTGACCT</u>CAGTTC

Numbers indicate the position of the nucleotides relative to the cap site of each gene.
The Tf PRI, Tf DRI and AT III-H regions were shown to be protected by liver nuclear extracts against the DNAse I cleavage in footprint experiments (5, and this paper). The highly homologous 10 nucleotide-long sequence is underlined.

nuclear factors, not detected in HeLa nuclear extracts (5). We have identified PRI as an hepatocyte promoter element and DRI as a positive regulatory element which modulates the promoter activity (Schaeffer *et al.*, submitted). Tf-PRI and Tf-DRI which contain each an identical 10 nucleotide-long sequence (5' TCTTTGACCT 3') (Table II) were previously shown to bind two different proteins (5). We name Tf-LF1 (Transferrin-Liver Factor 1) and Tf-LF2 (Transferrin Liver Factor 2) the liver factors binding respectively to the PRI and to the DRI *cis*-elements.

A DNA sequence homologous to the transferrin PRI and DRI *cis*-elements is present in the 5' flanking region of the human antithrombin III gene, and interacts with the liver nuclear factor Tf-LF1.

The common 10 nucleotide-long sequence present in the Tf PRI and DRI sites is almost identical to a DNA sequence (5' CCTTTGACCT 3') (Table II) present in a 328 bp fragment of the 5' flanking region of another human hepatic gene coding for antithrombin III (6). This 328 bp fragment (nucleotides -304/+24) was demonstrated to contain sufficient information for efficient expression of the gene in hepatoma cells but not in HeLa cells (6 and our unpublished results). We wondered if a 5' DNA sequence of the antithrombin III containing the 5' CCTTTGACCT 3' motif would be also a binding site for a nuclear protein. We thus performed DNAse I footprint experiments to detect a possible interaction between this motif and liver or HeLa (a human non-hepatic cell line) nuclear proteins. As

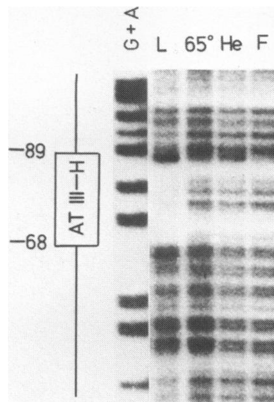


Figure 1 Footprint analysis of a human antithrombin III DNA fragment.
A *Sau*3AI-*Dra*I 174 bp fragment (6), 3' end labelled at position +24 (upper strand) was used in the binding reactions. A+G: chemical cleavage at purine residues; F: free DNA assay where no nuclear protein extract was added; He: experiments with HeLa nuclear protein extract; 65°: human liver nuclear protein extract heated at 65°C for 5 min before analysis; L: experiments with unheated human liver nuclear protein extract. Numbers alongside the autoradiograph indicate the positions relative to the cap site of the gene (6).

shown in Fig. 1 (lane L), (a) liver nuclear protein(s) is (are) able to protect a DNA region centered on the AT III 5' CCTTTGACCT 3' motif against DNAse I cleavage. The protected region extends from nucleotide -89 to nucleotide -68 on the upper strand (Figure 1, lane L) and from nucleotide -70 to nucleotide -88 on the lower strand (data not shown), and we shall refer to it as the AT III-H region. No interaction occurs between HeLa nuclear proteins and AT III-H (Fig. 1, lane He).

To determine whether the same or related proteins bind to this region and to the transferrin PRI or the transferrin DRI *cis*-elements, we performed heat inactivation experiments and gel retardation assays (9).

Heat inactivation assays followed by DNAse I footprint tests show that the factor binding to the AT III-H site is, like Tf-LF1 (5) inactivated after heating at 65°C for 5 minutes (Figure 1, lane 65°). In contrast, the Tf-LF2 protein resists inactivation upon heating to 65°C (5).

In gel retardation experiments, oligonucleotides containing the AT III-H and Tf-PRI binding sites give rise to DNA-protein complexes of identical electrophoretic mobilities (Figure 2). The relationship between the proteins

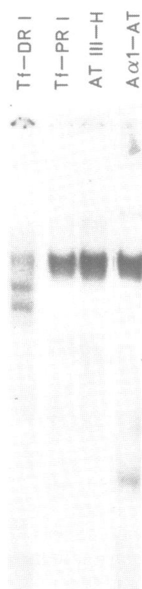


Figure 2 DNA-protein mobility shift assays using double strand oligonucleotides containing the Tf-PRI, the Tf-DRI, the AT III-H and the Aα1-AT binding sites (Table I) and rat liver nuclear extract. Experiments were performed as described in Materials and Methods. 6 μg of rat liver nuclear proteins were used in the binding reactions.

interacting with the AT III-H, Tf-PRI and Tf-DRI regions was further dissected by cross-competition assays with synthetic oligonucleotides. The experiments performed revealed that the AT III-H unlabelled oligonucleotide is able to strongly compete with the Tf-PRI DNA-protein complex formation. Figure 3A shows that a 10-fold molar excess of unlabelled AT III-H oligonucleotide is required to displace Tf-PRI DNA from its complex with protein, whereas a 40-fold molar excess of Tf-PRI is necessary to compete for its own DNA-protein complex. Figure 3B shows the same type of experiments carried out with the AT III-H oligonucleotide as a probe. We observe that a Tf-PRI unlabelled oligonucleotide is able to displace from a 40-fold molar excess on the AT III-H DNA from its complex with protein, while a 10-fold molar excess of AT III-H oligonucleotide competes very efficiently for the formation of the same complex. As shown in Figure 3C, DNAse I footprint experiments confirm these cross-competition results. The above experiments indicate a higher affinity of Tf-LF1 for the AT III-H binding site, than for Tf-PRI (compare lanes 1 to 4 with lanes 9 to 12 in Figures 3A and 3B, and lane 1 with 5 in Figure 3C). In contrast with the above mentioned results, it is noteworthy that no displacement of Tf-PRI (Figure 3A) or AT III-H (Figure 3B) DNA-protein complexes by the Tf-DRI unlabelled oligonucleotide is observed until a 200-fold molar excess of the competitor is attained.

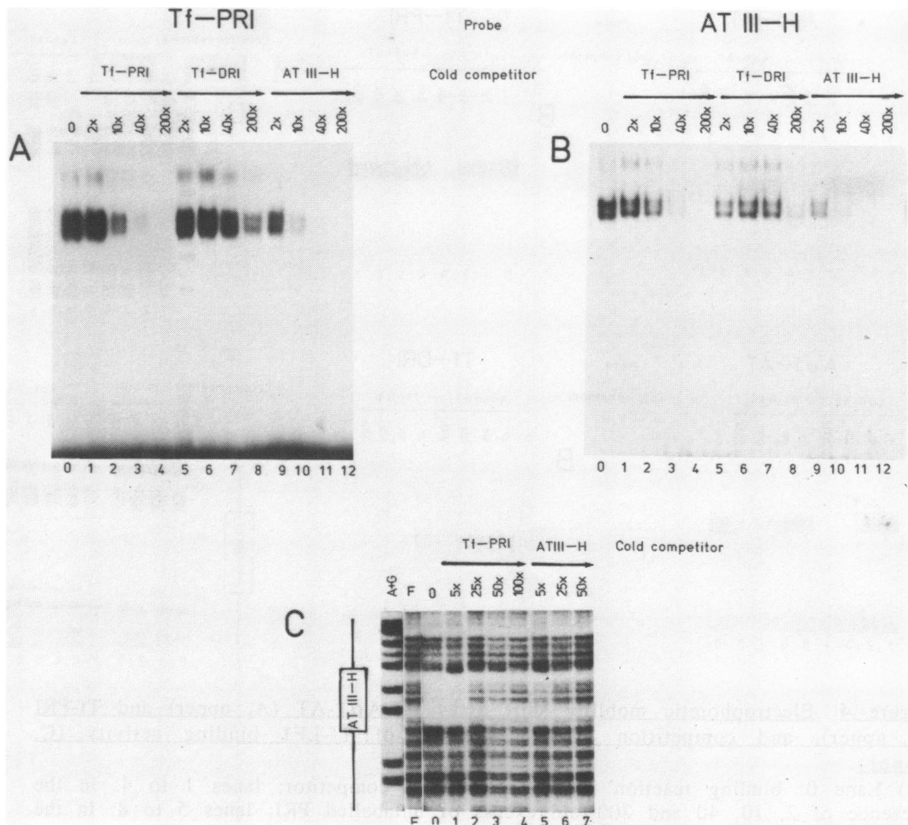


Figure 3 Electrophoretic mobility shift assay of Tf-PRI (A) and AT III-H (B).

5' end-labeled double strand oligonucleotides were incubated with 6 µg of rat liver nuclear protein extract, without or with various amounts of non-radioactive competitor oligonucleotides as noted. (A): lane 0: binding reaction in the absence of competitor; lanes 1 to 4: in the presence of 2, 10, 40 and 200-fold excess of unlabelled PRI; lanes 5 to 8: in the presence of 2, 10, 40 and 200-fold excess of non-radioacxtive DRI; lanes 9 to 12: in the presence of 2, 10, 40 and 200-fold excess of unlabelled AT III-H. (B): lane 0: binding reaction in the absence of competitor; lanes 1 to 12: as in (A).

Competition binding analysis of LF1 binding activity (C).

A *Sau*3AI-*Dra*I 174 bp fragment containing the AT III-H region, 3' end labelled at position +24 (upper strand) was used. A+G and F: as indicated in Fig. 1. Lane 0: experiment with human liver nuclear extract without non-radioactive competitor oligonucleotides; lanes 1 to 4: in the presence of 5, 25, 50 and 100-fold excess of unlabelled PRI; lanes 5 to 7: in the presence of 5, 25 and 50-fold excess of unlabelled AT III-H.

Taken together, the results presented in this paragraph lead to the plausible conclusion that the Tf-LF1 factor which binds to the Tf-PRI region is indeed the liver nuclear protein interacting with the AT III-H region.

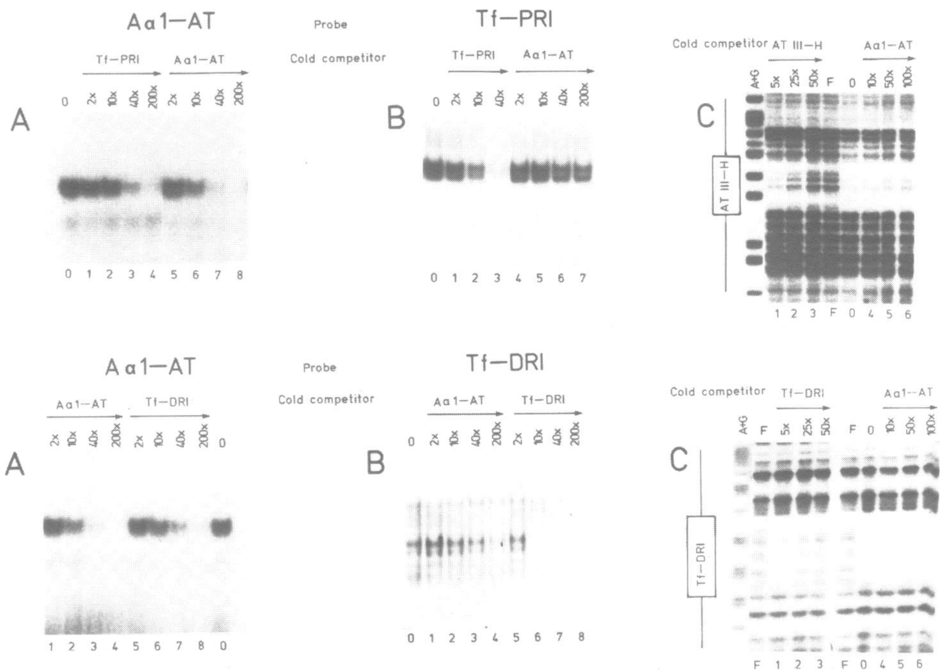


Figure 4 Electrophoretic mobility shift assay of Aα1-AT (A, upper) and Tf-PRI (B, upper), and competition binding analysis of Tf-LF1 binding activity (C, upper).

(A) Lane 0: binding reaction in the absence of competitor; lanes 1 to 4: in the presence of 2, 10, 40 and 200-fold excess of unlabelled PRI; lanes 5 to 8: in the presence of 2, 10, 40 and 200-fold excess of non-radioactive Aα1-AT.

(B): Lane 0: as in (A); lanes 1 to 3: in the presence of 2, 10 and 40-fold excess of unlabelled PRI; lanes 4 to 7: in the presence of 2, 10, 40 and 200-fold excess of non-radioactive Aα1-AT.

(C): A *Sau*3AI-*Dra*I fragment as in Fig. 1 was used. A+G and F: as indicated in Figure 1; lane 0: in the absence of competitor; lanes 1 to 3: in the presence of 5, 25 and 50-fold excess of unlabelled AT III-H; lanes 4 to 6: in the presence of 10, 50 and 100-fold excess of unlabelled Aα1-AT.

Electrophoretic mobility shift assay of Aα1-AT (A, bottom) and Tf-DRI (B, bottom), and competition binding analysis of Tf-LF2 binding activity (C, bottom).

(A): Lane 0: as in (A and B, upper); lanes 1 to 4: as lanes 5 to 8 in (A, upper); lanes 5 to 8: in the presence of 2, 10, 40 and 200-fold excess of unlabelled DRI.

(B): Lane 0: as in (A and B, upper and A, bottom); lanes 1 to 4: as lanes 5 to 8 in (A, upper); lanes 5 to 8: in the presence of 2, 10, 40 and 200-fold excess of unlabelled DRI.

(C): A *Hin*dIII-*Pst*I 210 bp fragment of the 5' flanking region of the human Tf gene (5, 8)., 3' labeled at position -405 (upper strand) was used. A+G and F: as in Figure 1; lane 0: in the absence of unlabelled competitor; lanes 1 to 3: in the presence of 5, 25 and 50-fold excess of cold DRI; lanes 4 to 6: in the presence of 10, 50 and 100-fold excess of unlabelled Aα1-AT.

The liver nuclear factor binding to the A domain of the α1-antitrypsin promoter (LF-A1) appears to be different from the liver factor interacting with the Tf-PRI or AT III-H binding sites.

In a recent paper, Hardon *et al.* (11) described the interaction of a liver specific *trans*-acting factor, named LF-A1 with the A domain (nucleotides -123/-103) of the α1-antitrypsin promoter (2). This factor was shown to bind also to the human apolipoprotein A1 promoter segment lying between nucleotides -225 and -195 (11). Methylation interference experiments allowed the authors to find in these two binding sites a six nucleotide-long consensus 5' TG$^{G}/_{A}$$^{C}/_{A}C^{C}/_{T}$ 3', where methylation of the homologous purines interferes with the binding of LF-A1 to its target sequences. One can then observe the presence of this 6 nucleotide-long consensus sequence in the 3' border of the Tf-PRI, Tf-DRI and AT III-H common motif 5' $^{T}/_{C}$CTTTGACCT 3'. This strongly suggests that either the Tf-PRI and the AT III-H, or the Tf-DRI regions can be targets of the described factor LF-A1. To determine whether LF-A1 is the same protein as Tf-LF1 factor we first performed gel retardation assays. As shown in Figure 2, the Tf-PRI, AT III-H and Aα1-AT oligonucleotides give rise with liver nuclear extracts to DNA-protein complexes of similar electrophoretic mobility. Cross-competition experiments in gel retardation assays, using labelled Aα1-AT oligonucleotide as a probe revealed that a 40-fold molar excess of the Tf-PRI (Figure 4A, upper) or of the AT III-H (data not shown) unlabelled oligonucleotides are able to compete for the Aα1-AT DNA-protein complex formation. The same type of experiments was carried out with labelled Tf-PRI oligonucleotide (Figure 4B, upper) or with a labelled AT III probe in DNAse I footprint experiments (Figure 4C, upper). Surprisingly, the unlabelled Aα1-AT oligonucleotide, even in a 100 to 200-fold molar excess, does not compete with the specific complexes formed between the Tf-PRI oligonucleotide (Figure 4B, upper) or the AT III-H fragment (Figure 4C, upper) with the Tf-LF1 protein. These results indicate that the Tf-PRI and the AT III-H regions contain binding motifs recognized by the liver nuclear factor LF-A1; on the contrary, the Aα1-AT promoter element seems not to be recognized by the Tf-LF1 liver protein.

A more precise analysis of the binding sites of the factors interacting with the DNA regions involved in our comparison was performed by methylation interference experiments. As shown in Figure 5, a 5' TGGACTTAGCCC 3' sequence containing the TG$^{G}/_{A}$$^{C}/_{A}C^{T}/_{C}$ motif at its 5' extremity seems to be the binding sequence of the Aα1-AT domain with the LF-A1 liver factor. Our results agree with those described by Hardon *et al.* (11). Figure 6 shows the methylation interference pattern performed on each strand of Tf-PRI and AT III-H

A α1-AT

A

1 2

C F C F

-111 ▶ ◀ -115

◁ -110

-117 ▶ ◁ -109

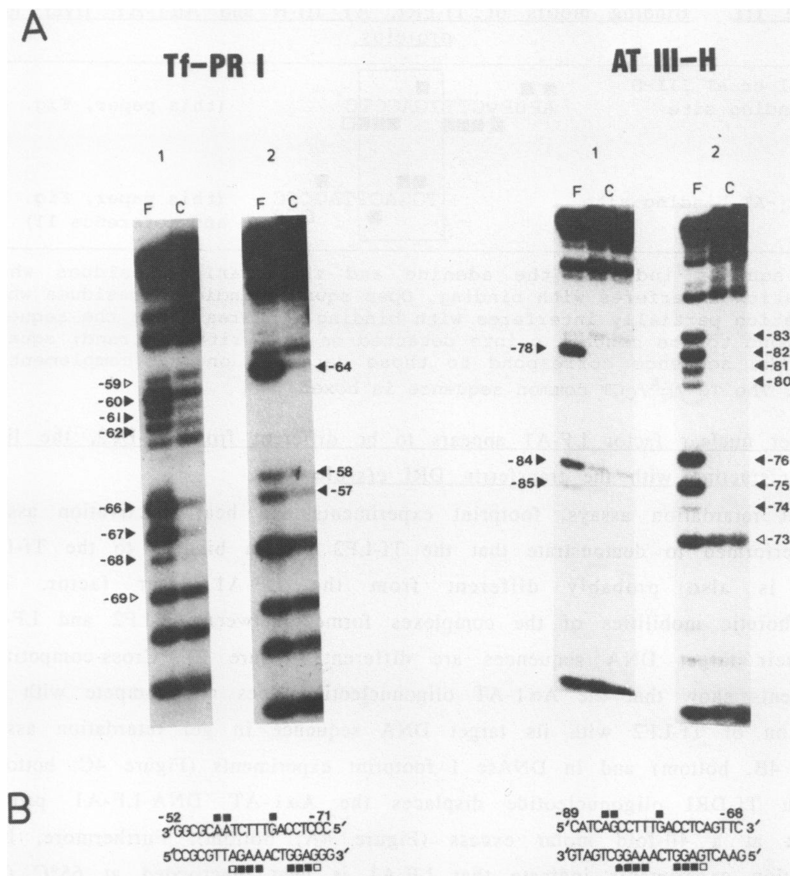
-118 ▶ ◀ -108

B

-119 -99
5'TGGACTTAGCCCCTGTTTGCT 3'
3'ACCTGAATCGGGGACAAACGA 5'

Figure 5 Methylation interference pattern of the binding of liver nuclear proteins on the Aα1-AT region.
(A) 1: upper strand; 2: lower strand; F: free DNA; C: DNA from the DNA-protein complex. Guanine and adenine residues at which methylation interferes with the binding are indicated by their positions in the DNA sequence.
(B) Summary of the methylation interference data. Solid squares indicate the residues whose methylation interferes with binding. Open squares indicate partial interference with binding.

oligonucleotides. This figure reveals that the contact points observed between the proteins and the analysed DNA regions are nearly identical; both DNA sequences present a common 5' $A^{A}/G^{T}/C$CTTTGACCTC 3' motif where methylation of the homologous purines interferes with the interaction of the liver protein with both binding sites. These results comfort our previous conclusion that the Tf-PRI and the AT III-H regions are targets for the same protein, Tf-LF1. On the other hand, as is clearly depicted in Table III, one can observe that this 5' $A^{A}/G^{T}/C$CTTTGACCTC 3' binding motif, although sharing with the Aα1-AT binding motif some essential guanines in its 3' border is different from it. The cross-



<u>Figure 6 Methylation interference pattern of the binding of liver nuclear proteins on the Tf-PRI and AT III-H regions.</u>
(A): 1, 2, F and C as in Figure 5.
(B): As indicated in Figure 5.
In order to facilitate the sequence comparison between the two fragments used, the upper and lower strands of Tf-PRI have been inverted.

competition results (Figure 4) can be explained by the fact that the 5' TG^{A}/G^{A}/CC^{T}/C 3' sequence must be an essential part of the binding motif to LF-A1 and is sufficient to bind the protein. On the contrary, this does not seem to be the case in the formation of a DNA-Tf-LF1 complex. Therefore Tf-PRI or AT III-H oligonucleotides are able to displace the Aα1-AT DNA-protein complex whereas the Aα1-AT oligonucleotide does not compete for the Tf-PRI or AT III-H-Tf-LF1 complex formation (Figure 4, upper A, B and C).

Table III. Binding motifs of Tf-PRI, AT III-H and Aα1-AT liver nuclear proteins.

Binding site	Sequence	Reference
PRI or AT III-H binding site	APuPyCTTTGACCTC	(this paper, Fig. 6)
Aα_1-AT binding site	TGGACTTAGCCC	(this paper, Fig. 5 and reference 11)

Solid squares indicate the adenine and the guanine residues whose methylation interferes with binding. Open squares indicate residues whose methylation partially interferes with binding. Squares above the sequence correspond to the contact points detected on the written strand; squares below the sequence correspond to those detected on the complementary strand. The $TG^{A}/_{G}{}^{A}/_{C}CT$ common sequence is boxed.

The liver nuclear factor LF-A1 appears to be different from Tf-LF2, the liver factor interacting with the transferrin DRI *cis*-element.

Gel retardation assays, footprint experiments and heat inactivation assays were performed to demonstrate that the Tf-LF2 protein binding to the Tf-DRI region is also probably different from the LF-A1 liver factor. The electrophoretic mobilities of the complexes formed between Tf-LF2 and LF-A1 with their target DNA sequences are different (Figure 2). Cross-competition experiments show that the Aα1-AT oligonucleotide does not compete with the interaction of Tf-LF2 with its target DNA sequence in gel retardation assays (Figure 4B, bottom) and in DNAse I footprint experiments (Figure 4C, bottom), although Tf-DRI oligonucleotide displaces the Aα1-AT DNA-LF-A1 protein complex at a 40-fold molar excess (Figure 4A, bottom). Furthermore, heat inactivation experiments indicate that LF-A1 is heat inactivated at 65°C (11) while Tf-LF2 resists inactivation upon heating at 65°C (5).

DISCUSSION

The available data concerning the tissue-specific expression of a gene, suggest that it may be the result of a unique combination of a limited number of *trans*-acting factors binding to defined *cis*-elements present in the DNA sequence of the gene (1). The comparison of the DNA sequences of *cis*-elements present in the 5' flanking region of different genes reveals highly homologous sequences. This suggests the existence of common regulatory elements, which might be the target of the same *trans*-acting factors. Thus, Kugler *et al.* (12) showed that promoter elements of the albumin, α1-antitrypsin (B domain) and β-fibrinogen genes all bind a common factor which may be identical to the hepatocyte nuclear factor 1 (HNF1) described previously (3). On the other hand, Costa *et al.* (13) identified a liver specific DNA-binding protein that recognizes

multiple nucleotide sites in regulatory regions of transthyretin, albumin and simian virus 40 genes. In contrast, it has been shown that in the case of the CCAAT-binding proteins a whole range of different proteins acts on similar albeit different sequences around the common CCAAT motif (14, 15, 16, 17).

The PRI and the DRI *cis*-elements of the human Tf gene are not only homologous with each other but also with the -89 to -69 region of the human AT III gene (referred to as the AT III-H region) and to the A domain of the human α1–antitrypsin promoter. We have already shown (5) that the Tf-PRI and the Tf-DRI regions bind two definitely distinct proteins, which are referred to in the present paper as Tf-LF1 and Tf-LF2. Here we demonstrate by gel retardation, cross-competition with synthetic oligonucleotides and methylation interference analysis that the AT III-H region and the Tf-PRI region appear to bind the same factor, Tf-LF1. In contrast, we also show that LF-A1, the factor that binds to the A domain of the α1-antitrypsin promoter and to the -221 to -195 DNA region of the apolipoprotein A I promoter is different from the Tf-LF1 liver factor on the one hand, and from Tf-LF2,the protein binding to the Tf-DRI region, on the other.

The results obtained by methylation interference experiments define more precisely the contact sites between the purines and the bound proteins; although the binding regions of the genes concerned in our study are homologous in sequence, they differ in their detail by the nature and the positions of the bases involved in the contacts (Table III). It is noticeable that in the case of the Tf-PRI and AT III-H regions bound to the Tf-LF1 factor, the methylation interference analysis shows with two exceptions, that identical bases are involved in the contacts with the liver protein; this may be related to the different affinities of Tf-LF1 for the two sequences (Figure 3). The binding motif of the Tf-PRI and AT III-H regions interacting with Tf-LF1 is 5' $A^{A}/_{G}{}^{T}/_{C}$CTTTGACCT 3'. This motif contains in its 3' border the six nucleotide-long consensus sequence 5' $TG^{G}/_{A}{}^{C}/_{A}C^{C}/_{T}$ 3' shown by Hardon *et al.* (11) to be an essential DNA region involved in the binding of LF-A1 to its target sites. This observation can explain the displacement of the Aα1-AT-LF-A1 DNA-protein complex by the Tf-PRI and AT III-H unlabelled oligonucleotides (Figures 4A and B, upper). On the contrary, the 5' $TG^{G}/_{A}{}^{C}/_{A}C^{C}/_{T}$ 3' sequence is clearly not sufficient to bind Tf-LF1, explaining the inability of unlabelled Aα1-AT oligonucleotide to compete for the Tf-PRI or AT III-H-Tf-LF1 complexes formation (Figures 4A and B, upper). The presence of a homologous sequence in the DNA binding sites of *trans*-acting factors seems thus not to be a sufficient condition *per se* to conclude that the proteins interacting with the concerned sequences are identical. It is interesting to underline the case of the CCAAT-binding proteins where sequences around the

CCAAT core motif must play a role in the discrimination between the CCAAT-boxes and the different CCAAT-binding proteins (14, 15, 16, 17).

As a result, we have shown in this paper that three different proteins present in liver nuclear extracts, Tf-LF1, Tf-LF2 and LF-A1, can bind to different subsets of 5' DNA regions containing homologous sequences.

COUP is a transcription factor interacting with the ovalbumin promoter sequence that has been now purified (18). The contact sites at which COUP binds to the DNA have been defined (19) and are quite similar to the DNA sites interacting with the Tf-LF1 liver factor. Surprinsingly, COUP has been detected in and purified from HeLa cell nuclear extracts. As stated before, no HeLa nuclear protein binds to the Tf-PRI region, target of the Tf-LF1 liver factor. Moreover, we have recently shown that Tf-PRI is a *cis*-hepatocyte promoter element, and that the Tf promoter is not active in HeLa cells (Schaeffer *et al.*, submitted). Whether these proteins, COUP and Tf-LF1, and even Tf-LF2 and LF-A1 are members of a common family must await the acquisition of the necessary structural information. Whatever the case, the latter is needed not only to establish a possible evolutionary relationship, but also to throw light on the mechanism by which *trans*-acting factors modulate gene expression.

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