Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7937–7941, November 1988
Biochemistry

Purified hepatocyte nuclear factor 1 interacts with a family of hepatocyte-specific promoters

(liver-specific expression/DNA-binding proteins/affinity purification/hepatitis B virus)

GILLES COURTOIS, SUSANNE BAUMHUETER, AND GERALD R. CRABTREE

Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

Communicated by James E. Darnell, Jr., July 26, 1988

ABSTRACT **During development cell types arise through the activation or repression of classes of specific genes. One hypothesis is that this phenomenon is realized by tissue-specific factors playing a role at the transcription level. Recently we have described a liver-specific nuclear protein, hepatocyte nuclear factor 1, that appears to be involved in the transcription of the fibrinogen and α_1-antitrypsin genes. In this report we describe the purification of hepatocyte nuclear factor 1 and demonstrate that it interacts with essential promoter regions of many liver-specific genes, including albumin, α-fetoprotein, and transthyretin. This finding suggests that hepatocyte nuclear factor 1 could be one factor necessary for establishing the liver phenotype. We also show that this protein binds to the promoter of the surface-antigen gene of the hepatitis B virus, a virus characterized by a high degree of hepatotropism.**

Transcription in eukaryotes involves the specific interaction of nuclear proteins with discrete DNA elements located in the promoter or enhancer region of genes (1, 2). Promoter regions cover, generally, the 100–200 base pairs (bp) upstream of the cap site, whereas enhancers can be found at large distances in the 5′ or 3′ regions of the genes they regulate or even within them (1, 2). In general, the full transcription activity of a gene implies the interaction between its promoter and enhancer(s) components through the various proteins they bind.

Some of the known DNA-binding proteins are restricted to a cell lineage (3–9). They interact with DNA sequences necessary for tissue-specific activation or repression of genes and constitute at least a part of the machinery eukaryotes use to synthesize proteins in a particular cell type. Transcriptional analysis of several hepatic genes (albumin, α-fetoprotein, and transthyretin) has revealed the presence of liver-specific promoters and/or enhancers (8–16). In several instances proteins binding to these regions have been described (8–10, 12, 17, 18). Analyzing the transcription of the fibrinogen genes we have localized in the β-chain promoter a functional sequence that binds a liver-specific nuclear protein, hepatocyte nuclear factor 1 (HNF1) (8). Because HNF1 also interacts with the gene for the α chain of fibrinogen and another liver-specific gene, α_1-antitrypsin, we suggested that HNF1 could be involved in developmentally regulated gene expression in the liver (8). The data presented here, which demonstrate that purified HNF1 interacts with a large family of hepatic genes, support this hypothesis.

MATERIALS AND METHODS

UV Cross Linking. The UV cross-linking experiments were done essentially according to Chodosh *et al.* (19). Seventy nanograms of a 28-bp double-strand oligomer, corresponding to the nucleotides −102 to −75 of the rat fibrinogen β-chain gene promoter, were hybridized with 3 μg of the complementary sequence 5′-TTTCCCTG-3′. By use of this primer the second strand was synthesized with the Klenow enzyme in the presence of 40 μCi of [α-^{32}P]dATP (1 Ci = 37 GBq), 500 μM of bromodeoxyuridine, and 500 μM of dCTP and dGTP. The full-length probe was purified on a 12% polyacrylamide gel, and 0.5 ng (50,000 cpm) were incubated with either 15 μg of a rat hepatoma cell line (Faza), liver nuclear extract, or 2 μg of heparin-Sepharose-purified liver extract for 45 min at room temperature. After 30 min of UV irradiation at room temperature with a Fotodyne UV transilluminator (λ emission = 310 nm), the mixture was analyzed on an 8% sodium dodecyl sulfate (SDS)/polyacrylamide gel after reduction with 100 mM dithiothreitol. After fixation the gel was stained with Coomassie blue to visualize the molecular mass markers and autoradiographed.

Purification of HNF1. Crude rat liver nuclear extracts were prepared essentially as described (8). Starting with 50 rats we usually obtained 2.5–3.5 g of nuclear proteins in 150 ml of buffer C100 [50 mM Hepes, pH 7.8/100 mM KCl/0.1 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/10% (vol/vol) glycerol]. The extract was applied to a 250-ml heparin-Sepharose column pre-equilibrated with buffer C100. The column was washed with 500 ml of buffer C100, and then the proteins were eluted with an 800-ml linear KCl gradient (100 ml/hr). Fractions of 4 ml were collected and analyzed for HNF1 by a mobility-shift assay (see below). The HNF1-containing fractions were pooled (volume, 30–40 ml; 200–300 mg), dialyzed against buffer C100, and chromatographed on an affinity column. The column was prepared using CNBr-activated Sepharose CL-4B and multimers of the −102- to −75-bp sequence of rat fibrinogen β-chain promoter (20). A coupling of ≈25 μg of DNA per ml of resin was achieved. Aliquots of 125 mg of heparin-Sepharose-purified HNF1 were incubated 10 min at 4°C with poly(dI·dC) (10 μg/ml) and then loaded on a 5-ml affinity column previously equilibrated with buffer C100. The column was washed extensively with buffer C100 and eluted sucessively with 20 ml of 0.2, 0.4, 0.6, 0.8, and 1 M KCl in buffer C (buffer C100 without KCl). Fractions containing HNF1 (0.4 M KCl) were diluted to 0.1 M KCl, incubated at 4°C for 10 min with poly(dI·dC) (2 μg/ml), and reapplied to the same affinity column. Elution of HNF1 from the column was done as described for the first pass. Analysis of protein was conducted according to Laemmli (21) with use of a silver-staining kit (Bio-Rad).

Denaturation–Renaturation of HNF1. The protocol described by Hager and Burgess (22) was followed with only minor modifications: 100 μg of bovine serum albumin was added as protein carrier and, after denaturation with guanidine hydrochloride, the denaturing agent was removed by dialysis against buffer C100.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HNF1, hepatocyte nuclear factor 1; SDS, sodium dodecyl sulfate; β 28, 28-bp double-stranded probe containing the HNF1-binding site of the rat gene encoding the β chain of fibrinogen.

Mobility-Shift Assay and DNase I Footprinting. These two techniques were conducted as described (8). When affinity-purified fractions were analyzed, 10 μg or 25 μg of bovine serum albumin were added as protein carrier for the mobility-shift assay and the DNase footprinting, respectively. The DNase footprinting probes were prepared as follows: The base-pair sequence from position −177- to +32 of the rat promoter of the gene for the α chain of fibrinogen, inserted into a chloramphenicol acetyltransferase-derived vector (JYM-CAT) between *Sph* I and *Hin*dIII (8), was labeled at the *Hin*dIII site. The sequence −291 to +1 of the rat β-fibrinogen promoter, inserted into JYM-CAT plasmid between *Sph* I and *Hin*dIII (8), was also labeled at the *Hin*dIII site. The *Dde* I fragment from −117 to +6 of the mouse α_1-antitrypsin promoter, inserted into the *Bam*HI site of pGEM-1, was labeled using the *Xba* I site in pGEM-1. This corresponds to the labeling of the coding strand of the α_1-antitrypsin promoter. A *Xba* I (−430) to *Bst*EII (+48) fragment derived from the rat albumin gene was labeled at *Bst*EII. A *Bam*HI (−202) to *Xba* I (−70) fragment derived from the mouse transthyretin gene was labeled at *Xba* I. A *Ssp* I (−133) to *Bst*EII (+11) fragment derived from the pre-S1 promoter (23) of the hepatitis B virus was labeled at *Bst*EIII. A *Hin*dIII (−323) to *Msp* I (+8) fragment derived from the rat α-fetoprotein promoter (24) was labeled at *Msp* I. In each case, labeling was done with the Klenow enzyme.

Materials. Synthetic oligomers were prepared by R. Belegage (Eli Lilly). A synthetic double-strand 23-mer corresponding to the base pairs from −63 to −41 of the rat albumin promoter was from M. Yaniv (Pasteur Institute, Paris). Mouse α_1-antitrypsin, rat albumin, and mouse prealbumin promoters were from R. Costa, D. Grayson, and J. Darnell (Rockefeller University). Rat α-fetoprotein genomic clone was from T. Sargent (National Institutes of Health), and a plasmid containing the hepatitis B virus genome was from A. Siddiqui (University of Colorado, Denver). Klenow enzyme was from Boehringer Mannheim; [α-^{32}P]dATP was from Amersham; heparin-Sepharose CL-6B, CNBr-activated Sepharose CL-4B, and poly(DI·dC) were from Pharmacia; guanidine hydrochloride and molecular weight markers were from Sigma; silver-staining kit was from Bio-Rad; bovine serum albumin was from Bethesda Research Laboratories; and restriction enzymes came from New England Biolabs.

RESULTS

UV Cross Linking of HNF1 to Its Binding Site. A cross-linking protocol was first devised to estimate the molecular mass of the liver-specific nuclear factor HNF1 directly from a crude nuclear extract. A bromodeoxyuridine-substituted probe, corresponding to the −102- to −75-bp region of the rat gene encoding the β chain for fibrinogen, was incubated with a nuclear extract prepared from Faza cells and irradiated under UV light to generate covalent links between the DNA and HNF1. The mixture was analyzed on a denaturing SDS/polyacrylamide gel and, as shown in Fig. 1*A*, a labeled band corresponding to a molecular mass of ≈110 kDa was detected (lane 1). In the presence of a large excess of unlabeled 28-mer this band disappeared, demonstrating that it represented the specific interaction between HNF1 and the probe (Fig. 1*a*, lane 2). In contrast, a smear covering the 40- to 60-kDa region was not competed with and most likely represented nonspecific interactions. A similar experiment was conducted with a nuclear extract derived from a rat liver, and the same 100-kDa protein was detected (Fig. 1*b*, lanes 1 and 2). When this extract was chromatographed through a heparin-Sepharose column (see below) and a fraction containing HNF1 was mixed with the probe, only the 110 kDa band was seen, further demonstrating that the 40–60 kDa smear corresponded to nuclear protein interacting nonspecifically with the probe (Fig. 1*b*, lane 3).

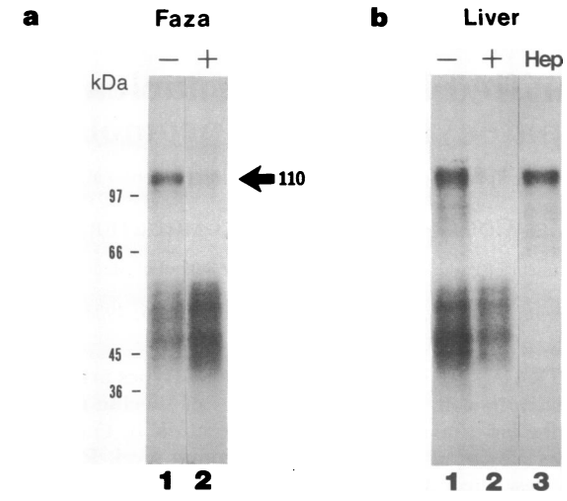

FIG. 1. UV cross-linking of HNF1 to its binding site. (*a*) Detection of HNF1 in a crude nuclear extract derived from the rat hepatoma cell line Faza without (lane 1) or with (lane 2) a 100-fold excess of unlabeled β 28 fragment. (*b*) Same analysis using a crude liver extract (lanes 1 and 2) or a heparin-Sepharose purified extract (Hep; lane 3). Arrow, HNF1.

Purification of HNF1. The purification of HNF1 was accomplished through two chromatographic steps. First, proteins of a crude extract from rat liver were loaded on a heparin-Sepharose column and eluted with a linear KCl gradient. To determine the fractions containing the factor HNF1 a mobility-shift assay was used with a 28-bp double-stranded probe (β 28) containing the HNF1-binding site of the rat gene encoding the β chain of fibrinogen. HNF1 activity was detected in fractions eluting between 0.35 and 0.4 M KCl (Fig. 2*a*). At this step an 8- to 10-fold purification was achieved, and the amount of protein recovered allowed us to use directly an affinity-chromatography procedure as a second step. This chromatography was conducted essentially according to Kadonaga and Tjian (20). The column was prepared by covalent coupling of multimers of β 28 to a CNBr-activated Sepharose CL-4B resin. The use of poly(dI·dC) as competitor for nonspecific interactions allowed purification to near homogeneity after two passes. As shown in Fig. 2*b*, the protein eluted from the affinity column with 0.4 M KCl, whereas most of the loaded protein (Fig. 2*c*, lane 1) appeared in the flow-through (data not shown).

The 0.4 M KCl fraction was analyzed on a denaturing SDS/acrylamide gel after silver straining (Fig. 2*c*, lane 3). A major band migrating at ≈88 kDa and two minor ones migrating at 55 and 45 kDa were seen in the preparation represented; another preparation gave us only the 88-kDa species (data not shown). The nature of the weaker bands at 55 and 45 kDa remained to be determined, but we had noticed in various preparations that a proteolytic degradation of HNF1 generated a lower-molecular mass form of ≈45 kDa, still retaining its binding activity (data not shown).

To definitely identify the protein in the purified HNF1 preparation, we carried out a denaturation–renaturation experiment (22). The proteins migrating around and between 90, 55, and 45 kDa were eluted from an SDS/acrylamide gel, precipitated with acetone, fully denatured with 6 M guanidine hydrochloride, and allowed to renature after removing the guanidine hydrochloride with dialysis. As shown in Fig. 2*d* the binding activity of HNF1 was detected mostly in the 90-kDa fraction (lane 3) but also in the 55- (lane 5) and 45-kDa (lane 7) fractions. The specificity of the interaction was confirmed using as competition either an excess of unlabeled

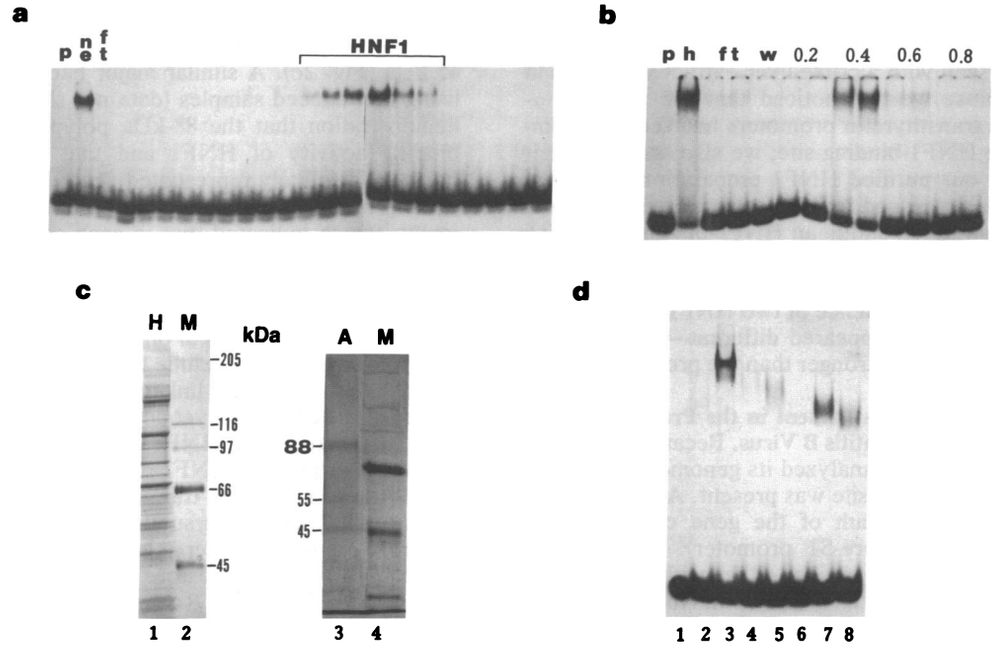

FIG. 2. Purification of HNF1. (*a*) Heparin-Sepharose chromatography. The fractions were analyzed for HNF1 using a mobility-shift assay and the probe β 28. p, Probe alone; ne, analysis of 5 μg of crude liver extract; ft, flow-through; HNF1, fractions containing HNF1. (*b*) Affinity chromatography. p, Probe β 28 alone; h, pooled heparin-Sepharose HNF1 fractions; ft, flow-through; w, wash; 0.2, 0.4, 0.6, and 0.8, fractions eluted at 0.2 M, 0.4 M, 0.6 M, and 0.8 M KCl, respectively. (*c*) Analysis of the purification using silver staining. M, molecular mass markers; H, pooled heparin-Sepharose HNF1 fractions (≈2 μg of protein); A, affinity chromatography. Approximately 70 ng of the 0.4 M KCl-eluted fraction (second pass) was analyzed. (*d*) Denaturation–renaturation experiment. Approximately 600 ng of affinity-purified HNF1 was loaded on a 7% SDS/polyacrylamide gel. Proteins migrating above 90 kDa (lane 2), in the 90-kDa region (lane 3) between 90 and 60 kDa (lane 4), in the 55-kDa region (lane 5), between 55 and 45 kDa (lane 6), in the 45-kDa region (lane 7), and below 45 kDa (lane 8) were eluted from the gel, renatured, and analyzed with the probe β 28 (lane 1) by use of a mobility-shift assay.

β 28 or of an unrelated oligomer (data not shown). We therefore assume that the 55- and 45-kDa proteins represent degradation products of HNF1, which is a polypeptide of ≈88 kDa.

The difference in size obtained from the UV crosslink and purification experiments could be attributed to the 28-bp DNA component that runs with the protein in the cross-linked sample. We favor this hypothesis because no difference in mobility-shift assay was seen between the heparin and affinity-purified fractions, therefore indicating that the 88-kDa band was not a degradation product of a 110-kDa form.

Purified HNF1 Interacts with a Family of Hepatic Genes. The purified protein HNF1 was used to footprint the promoter of the α and β chain of fibrinogen and α_1-antitrypsin genes (Fig. 3). The synthetic oligomer β 28, derived from the promoter for β-chain fibrinogen, had been used for HNF1 purification (see above), but we had previously shown that

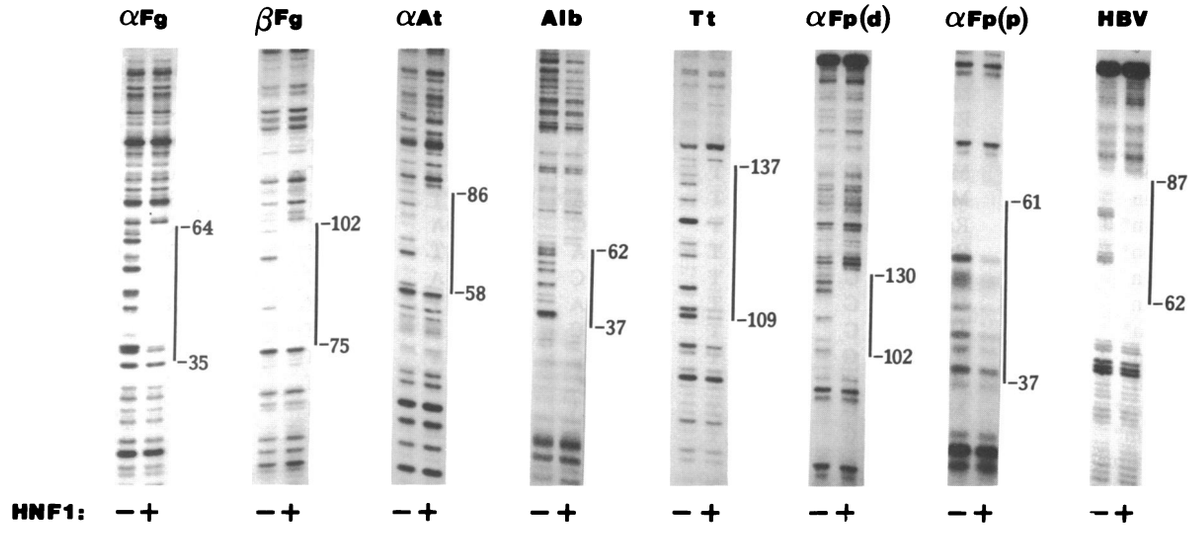

FIG. 3. Purified HNF1 interacts with a family of hepatic genes. The interaction of HNF1 with the fibrinogen α chain (αFg), fibrinogen β chain (βFg), α_1-antitrypsin (αAt), albumin (Alb), transthyretin (Tt), α-fetoprotein (αFp) and presequence 1 surface-antigen promoter of the hepatitis B virus (HBV) was analyzed with a DNase I footprinting assay. In each case ≈5 fmol of labeled coding strands were incubated without (−) or with (+) 10 ng (αFg, βFg, αAt, Alb, HBV) or 20 ng (Tt, αFp) of affinity-purified HNF1. αFp(d) and αFp(p) represent the distal and proximal HNF1-binding sites, respectively.

this factor, or a similar protein, also interacts with the α chain of fibrinogen and α_1-antitrypsin promoters (8). Footprints similar to those seen with a crude liver extract were seen in each case. Because we had noticed that the albumin, α-fetoprotein, and transthyretin promoters had sequence similarities with the HNF1-binding site, we also analyzed their interaction with our purified HNF1 preparation. Footprint analysis of the albumin and transthyretin promoters clearly demonstrated that they contain an HNF1-binding site localized between −62 and −37 bp and between −137 and −109 bp, respectively. In contrast, analysis of the α-fetoprotein promoter revealed the existence of two HNF1-binding sites. Their affinity for HNF1 appeared different—the distal site (−130 to −102 bp) being stronger than the proximal (−61 to −37 bp) site.

An HNF1-Binding Site Is Present in the Promoter for the Surface Antigen of the Hepatitis B Virus. Because hepatitis B virus infects the liver, we analyzed its genome to determine whether an HNF1-binding site was present. A good putative site was identified upstream of the gene coding for the surface-antigen protein (pre-S1 promoter). We therefore attempted to footprint this region, either with a crude liver extract or with the purified HNF1 protein. Analysis with the crude extract indicated protection of the putative HNF1 site, and this protection was specifically antagonized by β 28 (data not shown). A similar result was also seen with pure HNF1 (Fig. 3). The protected region extends from 62 to 87 bp upstream of the cap site for the pre-S1 mRNA.

DISCUSSION

We purified the liver-specific factor HNF1 to near homogeneity with the protocol recently described by Kadonaga and Tjian (20). The remarkable efficiency of this procedure is illustrated by the fact that after only one pass a pattern of protein similar to the one represented on Fig. 2*d* was already seen, suggesting an important degree of purification (data not shown). Considering that the three bands obtained after the second affinity pass correspond to HNF1 (see below), we estimate the overall purification of HNF1 from total nuclear proteins to be 85,000 fold.

The purified HNF1 preparation revealed a major band of 88 kDa and in some preparations two additional bands at 55 and 45 kDa (Fig. 2*c*). A similar major band (92 kDa) was seen using nonreduced samples (data not shown). The definitive demonstration that the 88-kDa polypeptide contained the binding activity of HNF1 and that the 55- and 45-kDa polypeptides likely represented degradation products of the same protein came from a denaturation–renaturation experiment, which indicated that in each case a binding activity specific for the probe β 28 was recovered. Because a similar mobility shift was seen in a crude extract and after renaturation of the 88-kDa polypeptide (data not shown), we assume that HNF1 is composed of only a single type of chain, but at this point we cannot exclude that it interacts with its binding site as a noncovalently linked homodimer (see below).

Because we have not yet developed an assay to measure *in vitro* the activity of HNF1, we are unable to determine whether our purified HNF1 preparations still have an effect on fibrinogen β-chain transcription or whether we have purified only the binding subunit of HNF1. If our preparation is active, it will be interesting to determine, for instance, whether HNF1 can restore transcription activity to the promoter for fibrinogen β chain in a nonhepatic extract. Such an effect has been seen with a pituitary-specific factor for the human growth hormone gene in a HeLa extract (25).

Purified HNF1 protein appears to interact with many hepatic gene promoters. Nevertheless, the definitive demonstration that exactly the same protein functionally interacts with all these promoters *in vivo* remains to be established. Preliminary experiments using probes containing the α-fibrinogen, α_1-antitrypsin, and albumin putative HNF1 site, in a mobility-shift assay, allowed the detection in each case of a liver-specific band that comigrated with the factor HNF1 (data not shown).

In almost every case where functional data are available, the HNF1-binding site is localized in an essential promoter region. We have already shown that the fibrinogen α and β-chain HNF1 sites appear necessary for promoter activity for those genes in hepatoma cell lines (8). Recently De Simone *et al.* (10) reported that clustered point mutations in the human α_1-antitrypsin promoter between positions −68

Table 1. HNF1-binding sequences

Promoter	Species	Sequence													
Fibrinogen α chain*	Rat	G	G	T	G	A	T	G	A	T	T	A	A	C	−47
Fibrinogen β chain*	Rat	G	T	C	A	A	A	T	A	T	T	A	A	C	−84
Fibrinogen β chain	Human	A	T	T	A	A	A	T	A	T	T	A	A	C	−77
Albumin	Mouse	G	T	T	A	A	T	G	A	T	C	T	A	C	−52
Albumin*	Rat	G	T	T	A	A	T	G	A	T	C	T	A	C	−53
Albumin	Human	G	T	T	A	A	T	A	A	T	C	T	A	C	−51
α-Fetoprotein	Mouse	G	T	T	A	C	T	A	G	T	T	A	A	C	−50
α-Fetoprotein	Mouse	G	T	T	A	A	T	T	A	T	T	G	G	C	−116
α-Fetoprotein*	Rat	G	T	T	A	C	T	A	G	T	T	A	A	C	−49
α-Fetoprotein*	Rat	G	T	T	A	A	T	T	A	T	T	G	G	C	−115
α-Fetoprotein	Human	G	T	T	A	C	T	A	G	T	T	A	A	C	−47
α-Fetoprotein	Human	G	T	T	A	A	T	T	A	T	T	G	G	C	−118
Transthyretin*	Mouse	G	T	T	A	C	T	T	A	T	T	C	T	C	−118
Transthyretin	Rat	G	T	T	A	C	T	T	A	T	T	C	T	C	−116
Transthyretin	Human	G	T	T	A	C	T	T	A	T	T	C	T	C	−116
α_1-Antitrypsin*	Mouse	G	T	T	A	A	T	—	A	T	T	C	A	T	−63
α_1-Antitrypsin*	Human	G	T	T	A	A	T	—	A	T	T	C	A	C	−63
Hepatitis B virus (pre S1)*	Human	G	T	T	A	A	T	C	A	T	T	A	C	T	−75
HNF1 consensus site: (18 sequences)		G_{17}	T_{17}	T_{17}	A_{17}	A_{12}	T_{16}	N	A_{15}	T_{18}	T_{15}	A_7	A_{11}	C_{16}	

A common DNA motif is recognized by HNF1. Only the 13-bp sequence that appears to be shared by the promoters analyzed is represented. The asterisks indicate promoters whose interaction with HNF1 is documented (this report and ref. 8). Nucleotides matching the HNF1 consensus sequence are in boldface. Numbers at right indicate the location of the HNF1 sites referenced to the last cytosine (or thymine) as reference.

and −64 or between −77 and −72 drastically affects the transcription of this gene. The albumin-binding site also appears to participate in transcription of the albumin gene (M. Yaniv, personal communication). Similarly a 5′-deletion analysis of the mouse α-fetoprotein gene has revealed that the region between residues −85 and −52, which destroys the proximal HNF1 binding site (see Fig. 3), results in a large drop of transcriptional activity (13). In contrast, removal of the distal site does not appear to significantly affect transcription of the α-fetoprotein gene. It is noteworthy that the affinity of HNF1 for the proximal site is fairly low compared with the other sites tested (see the legend for Fig. 3). This fact could support the observation of Jones *et al.* (26) for the binding of CTF/nuclear factor 1 to the β-globin promoter, which suggests that the localization and the context of a site, and not only its intrinsic affinity, might also be important parameters for its role in transcription. Functional information is limited for the promoter region of the transthyretin gene. Costa *et al.* (11) have demonstrated that the region from bp −151 to −108, which contains the HNF1-binding site (Fig. 3), is involved in the tissue-specific expression of this gene, but a more precise analysis is necessary. Finally, no functional information is available concerning the HNF1 site in the hepatitis B virus pre-S1 promoter. Nevertheless, its position, ≈55 bp upstream of a TATA box, strongly suggests that it might be involved in the transcription of the surface-antigen gene.

A compilation of the DNA sequences protected with HNF1 reveals a common structural motif (Table 1). Moreover, examination of these sequences in different species reveals that they are generally extremely conserved, suggesting their physiologic importance. We propose as a consensus site for HNF1 the sequence GTTAATNATTAAC (where N = A, C, T, G, or no nucleotide). This site contains an inverted repeat, indicating that HNF1 might bind as a homodimer generated by the noncovalent association of two 88-kDa polypeptide chains. A quick survey of other liver-specific genes suggests that an HNF1-binding site may exist in several of them, such as factor VIIIc (27), C-reactive protein (28), L-type pyruvate kinase (29) and play a role in their transcription.

The discovery that HNF1 interacts with many hepatic genes supports the hypothesis that tissue-specific expression of families of genes is achieved through the use of a limited number of DNA-binding proteins. An obvious advantage of this system would be the control of many genes solely by action on their common regulator(s). HNF1 could be one of these regulators in the differentiated hepatocyte. In support of such an idea, is our recent observation of a striking correlation between the presence of HNF1 and the differentiation state of the hepatocyte (30). Upon dedifferentiation, HNF1 disappears and is replaced by a protein of lower molecular mass with very similar sequence specificity; the relationship of this smaller protein to HNF1 remains to be elucidated. In contrast, a revertant cell line that has recovered hepatic functions contains a factor apparently identical to HNF1. Uncovering the functional role of HNF1 and its behavior during the differentiation process will, nevertheless, necessitate the cloning of this protein. Its purification, described in this report, represents a first step in this direction.

We thank R. Costa, D. Grayson, J. Darnell, T. Sargent, A. Siddiqui, R. Belegage, and M. Yaniv for generous gifts of plasmids or synthetic oligonucleotides. We also thank J. and R. Conaway for help during the preparation of the rat liver nuclear extracts, J. P. Shaw for review of the manuscript, and N. Leger for editorial assistance. This work was supported by National Institutes of Health Grants CA 39612 and HL 33942 to G.R.C.

1. Dynan, W. S. & Tjian, R. (1985) *Nature (London)* **316,** 774–778.
2. Maniatis, T., Goodbourn, S. & Fisher, J. A. (1987) *Science* **236,** 1237–1245.
3. Landolfi, N. F., Capra, J. D. & Tucker, P. W. (1986) *Nature (London)* **323,** 548–551.
4. Staudt, L. M., Singh, H., Sen, R., Wirth, T., Sharp, P. A. & Baltimore, D. (1986) *Nature (London)* **323,** 640–643.
5. Scheidereit, C., Heguy, A. & Roeder, R. G. (1987) *Cell* **51,** 783–793.
6. West, B. L., Catanzaro, D. F., Mellon, S. H., Cattini, P. A., Baxter, J. D. & Reudelhuber, T. L. (1987) *Mol. Cell. Biol.* **7,** 1193–1197.
7. Lefevre, C., Inagawa, M., Dana, S., Grindlay, J., Bodner, M. & Karin, M. (1987) *EMBO J.* **6,** 971–981.
8. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G. & Crabtree, G. R. (1987) *Science* **238,** 688–692.
9. Grayson, D. R., Costa, R. H., Xanthopoulos, K. G. & Darnell, J. E. (1988) *Science* **239,** 786–788.
10. De Simone, V., Ciliberto, G., Hardon, E., Paonessa, G., Palla, F., Lundberg, L. & Cortese, R. (1987) *EMBO J.* **6,** 2759–2766.
11. Costa, R. H., Lai, E. & Darnell, J. E. (1986) *Mol. Cell. Biol.* **6,** 4697–4708.
12. Costa, R. H., Lai, E., Grayson, D. R. & Darnell, J. E. (1988) *Mol. Cell. Biol.* **8,** 81–90.
13. Godbout, R., Ingram, R. & Tilghman, S. M. (1986) *Mol. Cell. Biol.* **6,** 477–478.
14. Ott, M.-O., Sperling, L., Herbomel, P., Yaniv, M. & Weiss, M. C. (1984) *EMBO J.* **3,** 2505–2510.
15. Gorski, K., Carneiro, M. & Schibler, U. (1986) *Cell* **47,** 767–776.
16. Pinkert, C. A., Ornitz, D. M., Brinster, R. L. & Palmiter, R. D. (1987) *Gene Dev.* **1,** 268–276.
17. Cereghini, S., Raymondjean, M., Carranca, A. G., Herbomel, P. & Yaniv, M. (1987) *Cell* **50,** 627–638.
18. Lichtsteiner, S., Wuarin, J. & Schibler, U. (1987) *Cell* **51,** 963–973.
19. Chodosh, L. A., Carthew, R. W. & Sharp, P. A. (1986) *Mol. Cell. Biol.* **6,** 4723–4733.
20. Kadonaga, J. T. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5889–5893.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Hager, D. A. & Burgess, R. R. (1980) *Anal. Biochem.* **109,** 76–86.
23. Ono, Y., Onda, H., Sasada, R., Igarashi, K., Sugino, Y. & Nishioka, K. (1983) *Nucleic Acids Res.* **11,** 1747–1757.
24. Nahon, J. L., Danan, J. L., Poiret, M., Tratner, I., Jose-Estanyol, M. & Sala-Trepat, J. M. (1987) *J. Biol. Chem.* **762,** 12479–12487.
25. Bodner, M. & Karin, M. (1987) *Cell* **50,** 267–275.
26. Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J. & Tjian, R. (1987) *Cell* **48,** 79–89.
27. Gitschier, J., Wood, W. I., Goralka, T. M., Wion, K. L., Chen, E. Y., Eaton, D. H., Vehar, G. A., Capon, D. J. & Lawn, R. M. (1984) *Nature (London)* **312,** 326–330.
28. Woo, P., Korenberg, J. R. & Whitehead, A. S. (1985) *J. Biol. Chem.* **260,** 13384–13388.
29. Cognet, M., Lone, Y. C., Vaulont, S., Kahn, A. & Marie, J. (1987) *J. Mol. Biol.* **196,** 11–25.
30. Baumhueter, S., Courtois, G. & Crabtree, G. R. (1988) *EMBO J.* **7,** 2485–2493.