LFB1/HNF1 acts as a repressor of its own transcription

Giulia Piaggio*, Licia Tomei¹, Carlo Toniatti¹, Raffaele De Francesco¹, Jutta Gerstner^{2,†} and Riccardo Cortese¹

Istituto 'Regina Elena' Centro Ricerca Sperimentale, Laboratorio Oncogenesi Molecolare, via Delle Messi d'Oro 156, 00158 Rome, ¹IRBM— Istituto di Ricerche di Biologia Molecolare P. Angeletti, via Pontina Km 40.600, 00040 Pomezia, Rome, Italy and ²EMBL, European Molecular Biology Laboratory, Meyerhofstraße 1, 6200 Heidelberg, Germany

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ABSTRACT

LFB1/HNF1 is a hepatocyte-enriched *trans*-activator involved in the regulation of many liver-specific genes. We report the cloning and characterization of a rat genomic DNA fragment containing about 3.5 kb of the LFB1/HNF1 gene 5'-flanking region. This DNA segment is capable of directing the liver-specific expression of a reporter gene in transfection assays. More interestingly, the basal activity of the LFB1/HNF1 promoter in cultured hepatoma cell lines is down-regulated by exogenously added LFB1/HNF1 protein itself. The ability to repress transcription starting from its own promoter requires the integrity of the N-terminal LFB1/HNF1 DNA-binding domain. Contrary to the expectations, *in vitro* binding experiments failed to demonstrate any specific and functional interaction of purified LFB1/HNF1 with the –3.5 kb promoter sequence. In addition to the DNA-binding domain, a 60 aa region contained in the C-terminus of the protein and distinct from the previously characterized activation domains, is also required for the repressing function.

INTRODUCTION

The liver provides a useful model system in which to study the mechanisms that govern the differential expression of tissue-specific genes (1). Several cDNAs coding for factors that play an important role in controlling the hepatocyte-specific expression have recently been cloned. They include the Liver Factor B1/Hepatocyte Nuclear Factor 1 (LFB1/HNF1; 2,3) and the related factor LFB3/vHNF1 (4,5), the *Drosophila fork head* homologue HNF3 (6), HNF4, a member of the steroid hormone receptor superfamily (7) and the leucine-zipper containing factors C/EBP (8) and DBP (9). The interaction of these transcription factors with a given array of *cis*-acting elements on the promoter region of liver-specific genes modulates their expression rate. This interaction may in fact activate or repress promoter activity according to the stage of development, hormonal induction or pathological condition (10). The comprehension of the molecular

mechanisms underlying hepatocyte-specific transcriptional regulation may provide insight into the events leading to liver development and differentiation.

LFB1/HNF1 was initially identified as a hepatocyte factor interacting with the promoter regions of many liver-specific genes (10,11). Although its expression was shown not to be limited to hepatic cells, both *in vivo* and *in vitro* studies have confirmed this protein to be one of the major determinants of the liver phenotype (5,12–14).

LFB1/HNF1 binds as a dimer to specific palindromic sites (2,11,15). The functional architecture of the LFB1/HNF1 protein has been extensively studied in our laboratory. A protein fragment that contains the N-terminal 281 residues of LFB1/HNF1 binds to DNA with affinity and specificity comparable to those of the native protein (2). Further dissection of this minimal DNA-binding domain revealed a unique tripartite DNA binding structure that includes an unusually long homeodomain, a region related to the POU-specific A-box, and a short N-terminal dimerization domain (15,16). The C-terminus of the molecule (aa 282–628) contains at least three regions that have been shown to be indispensable for the transcription activation function: the serine-rich ADI (Activation Domain I, aa 547–628), the proline-rich ADII (aa 282–318) and the glutamine-rich ADIII (aa 440–506) (15,17,18).

The cloning and functional characterization of the rat and mouse LFB1/HNF1 promoter region was reported (19, 20). It has been shown that the activity of this promoter in cultured hepatoma cell lines can be enhanced by co-transfection with cDNAs coding for the hepatocyte-enriched *trans*-activators HNF4 and HNF3 (19, 20).

Autoregulation is a common mechanism through which the correct balance between transcription factors is established and maintained during the developmental stages of the cells or in response to external stimuli (21–23). Conflicting results have been reported about the role of LFB1/HNF1 in regulating the transcriptional activity of the promoter of its gene (24–26). In this paper we show evidences of a negative autoregulatory loop, through which LFB1/HNF1 negatively controls transcription

* To whom correspondence should be addressed

† Present address: Leica Vertrieb GmbH, Lilienthalstraße 39–45, D-6140 Bensheim, Germany

driven from a 3500 bp fragment of its own promoter. We show that an intact DNA-binding domain and a region contained in the C-terminal moiety of the protein are both required for the autorepressing activity of LFB1/HNF1, even though direct binding to the promoter sequence could not be demonstrated.

MATERIALS AND METHODS

Isolation of the LFB1/HNF1 promoter region

2.5×10⁶ recombinant phage plaques from an amplified EMBL-3 rat genomic library were screened by hybridization with a labelled DNA fragment encompassing positions 1/1840 of the LFB1/HNF1 cDNA (λ18; 2). Plating of the phages and duplicate lifts were done by standard procedures (27). Lifts were presoaked in 6×SSC (0.9 M NaCl, 0.09 M Na Citrate), 1×Denhardt's, 100 μg/ml salmon sperm DNA and hybridized in the same buffer containing the labelled probe for 16 h at 65°C. Filters were washed three times in 6×SSC, 0.1% SDS and once in 0.2×SSC, 0.1% SDS for 30' at 65°C. The DNA was purified from one positive clone (λ4-5) (28) after tertiary screening and characterized by restriction digestion revealing a 30 kb long insert. Southern blot analysis identified a 3.7 kb *SacI*–*SacI* fragment, hybridizing with the λ18 probe. This fragment was subcloned in the *SacI* site of the pUC19 vector (construct S2/12) and sequenced.

DNA sequencing

To obtain the complete sequence of the LFB1/HNF1 5'-flanking region, several overlapping fragments from the 3.7 kb insert of the S2/12 construct (*SacI*–*EcoRI*, positions 1/1271; *PstI*–*PstI*, positions 220/2205 and 2205/3760; *HincII*–*EcoRI*, positions 457/1271; *AluI*–*AluI*, positions 691/750 and 1316/1885; *SmaI*–*SacI*, positions 3138/3772; *Sau3AI*–*Sau3AI*, positions 2561/3005) were subcloned into pUC19. Each insert was sequenced on both strands by the dideoxy method (29), using forward and reverse M13 primers flanking the cloning sites, or internal synthetic primers.

Plasmid constructions

The plasmid pE8U-3.5CAT, used in the transfection experiments, was obtained by sub-cloning the 3598bp *SacI*/*SpeI* fragment (positions –3510/+88, relatively to the transcription starts site) from the S2/12 plasmid, in the *SmaI* site of the pE8UCAT polylinker (30), upstream of the chloramphenicol acetyltransferase (CAT) gene, after filling-in with Klenow polymerase. The strong transcription terminator from the *mos* gene, contained in the pE8UCAT vector, minimizes artifactual readthrough transcription from cryptic vector promoters.

The RSV-LFB1 construct, expressing the full-length LFB1/HNF1 protein under the control of the Rous Sarcoma Virus LTR promoter (RSV) in the pGM-4 vector has been already described (31). To construct the plasmid H2-HNF4, the *BamHI*–*BamHI* fragment from pLEN4S (kindly provided by J.E.Darnell), containing the full-length HNF4 protein, was filled-in with Klenow and cloned into the *NheI*–*XhoI* sites, both blunted, of the pH2 expression vector (5). For the construction of the hybrid protein TAT/LFB1, a DNA fragment coding for the aminoacid region from A₂₁ to F₃₈ of the HIV TAT protein has been obtained by filling-in of two partially complementary single stranded oligos. This fragment has been cloned into the *NcoI*–*SphI* sites, both blunt-ended with Klenow enzyme, of the

LFB1/HNF1 mutant Δ1 in the BlueScript vector (15; bsTAT/LFB1). The Δ1 vector was previously mutagenized to destroy a second *NcoI* site present in the LFB1/HNF1 coding sequence. The *NheI*–*BglII* fragment from bsTAT/LFB1, containing the TAT/LFB1 chimera in the context of full-length LFB1/HNF1 protein, was subsequently cloned into the *XbaI*–*BamHI* sites of pGM-4 (RVS-TAT/LFB1).

The construction of RSV-GAL4/LFB1 and the LFB1/HNF1 C-terminal deletions, including the RSV-LFB1ΔC281 which expresses the LFB1/HNF1 DNA binding domain, has been already described in detail (18).

Cell cultures, DNA transfections and CAT assays

HepG2 and HeLa cell lines were cultured in Dulbecco's modified Eagle's medium + 10% fetal calf serum (FCS) at 5% CO₂. H4 II and H5 were cultured in a 1:1 mixture of F12/NCTC-135 media (GIBCO/FLOW) + 10% FCS at 7% CO₂.

DNA transfections were performed with the calcium phosphate precipitation technique as previously reported (32). Each cell line was transfected with an aliquot of a precipitate prepared in duplicate. In each experiment 200 ng of CMV-luciferase plasmid were transfected as an internal control for transfection efficiency. Cells were harvested 36 h after transfection; CAT activity was assayed in whole-cell extracts according to Gorman *et al.* (33) and normalized to the luciferase activity present in the same extract, measured as described (34).

Western immunoblot

Nuclear extracts from HepG2 cells, not transfected or transfected, were prepared as described (18) and separated on a 10% PAGE/SDS gel. The gel was then electroblotted on nitrocellulose membrane and subsequently immunostained using a 1:1 mixture of a polyclonal antibody generated against the last 30 aminoacids at COOH-terminal and a polyclonal antibody generated against the DNA binding domain of the LFB1/HNF1 protein.

RESULTS

LFB1/HNF1 acts as a negative regulator of its own promoter

In order to isolate the LFB1/HNF1 promoter sequence, a DNA segment spanning the 5' region of the rat LFB1/HNF1 cDNA (λ18; 2) was used as a probe to screen a rat genomic EMBL-3 library. A positive clone (λ4-5) containing a 3.7 kb *SacI*–*SacI* insert was isolated and sequenced. The isolated DNA sequence (not shown) contained 185 nucleotides of the LFB1/HNF1 cDNA coding sequence up to the previously characterized *SacI* site (2) and about 3.5 kb of the 5'-untranslated region.

To test the competence of this DNA segment to direct tissue-specific transcription the 5' flanking region of the LFB1/HNF1 gene from nucleotide –3515 to +88 (relatively to the transcription start site) was linked to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene in the pE8UCAT eukaryotic expression vector (30). The activity of the resulting construct, called pE8U-3.5CAT, was tested in transfection experiments using cell lines of hepatic (HepG2) and non hepatic origin (HeLa). The results indicate that the –3.5kb LFB1/HNF1 promoter segment is capable of directing efficient transcription in human hepatoma cells (HepG2, Fig.1A), in which the endogenous LFB1/HNF1 gene is expressed, but not in cells, which do not contain any detectable LFB1/HNF1 activity (HeLa cells; data not shown). This finding suggests that the –3.5 kb

Figure 1. LFB1/HNF1 represses its own promoter activity. **A)** HepG2 cells were transfected with 10 µg of the pE8U-3.5CAT plasmid carrying the -3.5 kb LFB1/HNF1 promoter fragment and increasing amounts (specified on the figure) of a construct expressing the full length LFB1/HNF1 protein under the control of the Rous Sarcoma Virus LTR promoter (RSV-LFB1). Data in the right panel are expressed as percentage of the LFB1/HNF1 promoter efficiency in the absence of cotransfected LFB1/HNF1 expression plasmid. A representative CAT assay is shown in left panel. **B)** Transfection in HepG2 cells with the 10 µg of the LFB1/HNF1-responsive construct CRP-CAT and the specified amounts of RSV-LFB1. The efficiency of *trans*-activation of the CRP promoter by LFB1/HNF1 is reported as fold increase over its basal activity. **C)** CAT assay from HepG2 cells transfected in with 10 µg of the RSV-CAT construct and the specified amount of RSV-LFB1. CAT activities were measured as described in Materials and Methods 36 hours after transfection. The values in panel A and panel B are the mean of those obtained from 3 independent experiments.

5'-flanking region of the LFB1/HNF1 gene contains *cis*-acting elements sufficient for its cell-type specific expression at least *ex vivo*.

In order to test the possibility that an autoregulatory loop might be involved in the control of the LFB1/HNF1 gene expression, pE8U-3.5CAT was used as reporter plasmid and transfected together with increasing amounts of an LFB1/HNF1 expression construct (RSV-LFB1, 31) in both HeLa and HepG2 cells. No CAT activity was detectable when HeLa were used as recipient cells, even at the highest concentration of the cotransfected LFB1/HNF1 expression plasmid (data not shown). This result indicates that LFB1/HNF1 is not capable of acting as a positive regulator of its own transcription. On the contrary, increasing amounts of RSV-LFB1 incrementally decreased the -3.5 kb promoter activity in HepG2 cells (Fig.1A). At the highest concentration used, more than 80% reduction of the promoter activity was observed. In a series of cotransfection experiments performed in parallel we confirmed that RSV-LFB1 directs the synthesis of a stable protein, capable of transactivating an

Figure 2. *Trans*-inhibition of the LFB1/HNF1 promoter activity in HeLa cells. The pE8U-3.5CAT reporter plasmid (10 µg) was transfected in HeLa cells together with 10 µg of an expression vector encoding for the HNF4 protein (H2-HNF4) and the indicated amounts of RSV-LFB1. Cells were collected 36 h after transfection and CAT activities were measured. Top panel: the average results from 2 independent experiments are shown. Values (in arbitrary units) are expressed as the amount of CAT conversion corrected by the transfection efficiency as determined by the luciferase activity in the same cell extracts. Bottom panel: a representative CAT assay is shown.

LFB1/HNF1-responsive promoter (CRP-CAT; 5) both in HepG2 (Fig.1B) and HeLa cells (5,18). It has to be pointed out that no repression could be observed under the same experimental conditions on different promoters, such as RSV (Fig.1C) and CMV (not shown), which do not contain LFB1/HNF1 DNA-binding sites.

It has been shown that the liver-specific transcription factor HNF4 is a major determinant of the cell-specific expression of the LFB1/HNF1 gene (19,20). Consistent with these reports, we have found that HNF4 is also able to transactivate the -3.5kb LFB1/HNF1 promoter fragment: a strong CAT gene expression from this promoter fragment has been obtained in HeLa cells in cotransfection experiments with increasing amounts of the HNF4 protein expressed under the control of the H2 promoter (H2-HNF4; Fig.2). The possibility to obtain the expression of the LFB1/HNF1 promoter in non-hepatic cells upon the cotransfection of the HNF4 protein, has been used to verify whether the autorepression activity of LFB1/HNF1 was restricted to cells of hepatic origin. We therefore performed experiments in HeLa cells in which the expression of the -3.5kb LFB1 promoter was allowed by the cotransfection of H2-HNF4 and then challenged with increasing amounts of RSV-LFB1. The results, reported in Fig. 2, show that LFB1/HNF1 is able to *trans*-inhibit its own promoter expression even in cells where the gene is not normally expressed, suggesting that the observed autorepression is not a tissue-specific phenomenon.

Figure 3. A) Trans-repression of the LFB1/HNF1 promoter activity by a dimerization domain substitution mutant of the LFB1/HNF1 protein. Increasing amounts of the RSV-TAT/LFB1 construct, expressing a hybrid protein bearing the dimerization domain of the TAT protein instead of that of LFB1/HNF1, were transfected in HepG2 cells together with 10 µg of the pE8U-3.5CAT reporter plasmid. The -3.5 kb LFB1/HNF1 promoter activity in the presence of the TAT/LFB1 chimera is reported as percentage of its basal expression. B) Transfection in HepG2 cells with 10 µg of the LFB1/HNF1-responsive construct CRP-CAT and the specified amounts of RSV-TAT/LFB1. CAT activities were measured as described 36 hours after transfection.

The LFB1/HNF1 dimerization domain is not directly involved in the LFB1/HNF1 promoter autoregulation

As a first step toward the elucidation of the molecular mechanisms underlying the LFB1/HNF1 promoter autoregulation, we decided to determine which portions of the protein are necessary to confer transcription repressing properties.

The dimerization domain of LFB1/HNF1 has been mapped to the N-terminal 32 amino acid residues of the protein (15). This region is crucial for the activity of the protein. In fact, LFB1/HNF1 binds efficiently and specifically to DNA only as a dimeric molecule. It has also been shown that the dimerization domain of the protein is responsible for the formation of heterodimers with the transcription factor LFB3/vHNF1 (5,35). The same region has been shown to be involved in the interaction with another protein, DCoH, which is present in several tissues and cell lines (36). It is therefore conceivable that, through its dimerization domain, the exogenously-added LFB1/HNF1 could sequester a related transcription factor or a cofactor that is essential for the LFB1/HNF1 promoter activity thus repressing its expression.

In previous studies we have shown that the dimerization domain of LFB1/HNF1 behaves as an independent module and can be substituted with dimerization domains derived from other proteins (15,16). The resulting chimeric molecules still bind *in vitro* to the LFB1/HNF1 recognition sequence (37). To establish whether the negative effect of LFB1/HNF1 on the expression of its own promoter is mediated by its dimerization domain, a substitution mutant was constructed, in which the 32 N-terminal residues of LFB1/HNF1 were changed with a 16 aminoacid sequence responsible for the dimerization of the HIV TAT protein (38,40). The resulting hybrid protein (TAT/LFB1) binds as a homodimer to the LFB1/HNF1 recognition sequence and stimulates LFB1/HNF1-dependent promoters, but is neither able to heterodimerize with the LFB1/HNF1 wild-type molecule, nor to interact with the DCoH protein (L.T. and R.D.F., unpublished results). If the autorepression of LFB1/HNF1 of its own promoter activity depended on its participation in a heteromeric complex involving protein-protein interactions through the dimerization domain, we expected that the TAT/LFB1 chimera would not be able to mediate autorepression. On the contrary, we found that the TAT/LFB1 hybrid protein, expressed under the control of the RSV promoter, retains the ability to repress transcription from the -3.5 kb LFB1/HNF1 promoter (Fig. 3A), while is able to activate an LFB1/HNF1 dependent promoter (CRP-CAT; Fig. 3B). The promoter repression by TAT-LFB1 is slightly less potent than that by the wild type protein. This could depend on lower DNA binding affinity or on a decreased stability of the TAT-LFB1 dimer *in vivo*, even though we have observed no significant impairment of DNA binding or dimerization properties *in vitro*.

On the basis of these results, we can reasonably exclude that LFB1/HNF1 mediates repression of its own promoter by sequestering a critical protein factor through its dimerization domain. In addition, this experiment proves that the repression can be attributed to the homodimeric form of LFB1/HNF1.

Regions within both the DNA-binding domain and the C-terminal half of the LFB1/HNF1 protein are required for repression

In previous studies it was shown that the N-terminal 281 aminoacids of LFB1/HNF1 are necessary and sufficient for sequence-specific DNA binding but lack the ability to *trans*-activate LFB1/HNF1-dependent promoters (2,15).

The negative effect of LFB1/HNF1 on the activity of its own promoter could be due to a competition for binding to a common or overlapping site between LFB1/HNF1 and an unknown, but more potent, activating factor. In this case, one would expect that the isolated DNA-binding domain should still be able to repress transcription from the LFB1/HNF1 promoter. As shown in Fig. 4A, increasing amounts of transfected LFB1/HNF1 DNA-binding domain have no effect on transcription of the CAT gene starting from the -3.5kb LFB1/HNF1 promoter, even though a stable protein is expressed in transfected cells as assayed by Western blot (Fig.4B).

These observations raised the possibility that the repressing properties of LFB1/HNF1 might be attributed to the C-terminal portion of the protein, which contains the activation domains (15,18). To test this hypothesis, a hybrid protein was constructed in which the region of LFB1/HNF1 from aa 282 to the carboxyl end was fused downstream to the DNA-binding domain of the yeast transcription factor GAL4 (GAL4/LFB1). This chimera

Figure 4. The DNA binding domain and the C-terminus of LFB1/HNF1 are not *per se* sufficient for repression. **A)** HepG2 cells were transfected with 10 μ g of the pE8U-3.5CAT reporter plasmid and an expression vector coding for the DNA binding domain (aa M₁–L₂₈₁) of LFB1/HNF1 (RSV-LFB1 Δ C281) or for a hybrid protein containing the DNA binding domain of GAL4 and the C-terminal region (aa M₂₈₃–Q₆₂₈) of LFB1/HNF1 (RSV-GAL4/LFB1). The results obtained by cotransfecting 1 μ g of the expression constructs are reported; the values, expressed as percentages of the basal –3.5 kb LFB1/HNF1 promoter efficiency, are the average of 3 independent experiments. **B)** Western blot on nuclear extracts from HepG2 cells (lane 1) and from HepG2 cell transfected with 10 μ g of pE8U-3.5CAT reporter plasmid and 1 μ g of the expression vector coding for the DNA binding domain of LFB1/HNF1 (RSV-LFB1 Δ C281) (lane 2). The bands corresponding to the endogenous LFB1/HNF1 protein (LFB1 FI) and to the transfected LFB1/HNF1 binding domain (LFB1bd) are pointed by arrows.

is able to activate transcription from a GAL4 responsive promoter in cultured hepatoma cells (18), but is unable to repress the LFB1/HNF1 promoter activity in HepG2 cells (Fig. 4A). It is worth noting that both the LFB1/HNF1 DNA binding domain and GAL4/LFB1 proteins accumulate in the nuclei of transfected cells as previously reported (18).

The results just described indicate that both the N-terminal DNA-binding domain and the C-terminal fragment of the molecule, although *per se* not sufficient, are required in order to observe the self-inhibiting activity of LFB1/HNF1.

A 60 amino acid-long region in the C-terminus of LFB1/HNF1 is required for full promoter-specific transcriptional inhibition

We next asked whether the integrity of the C-terminus portion of the protein is required for full inhibitory transcription properties. In particular, we wished to establish whether the domains required for the activation of liver-specific promoters *in vivo*, i.e. ADI (aa 546–628) and ADIII (aa 440–506) (17,18), are also involved in autorepression. For this purpose a set of C-terminal deletion mutants were tested in cotransfection experiments together with the pE8U-3.5CAT reporter plasmid. All these LFB1/HNF1 derivatives were previously shown to accumulate in the nuclei of transfected cells at a level comparable to that of the wild type protein (18). The results, shown in Fig. 5, indicate that both ADI and ADIII can be removed (see mutant Δ C440) without affecting the autorepression activity of the protein. Upon further deletion the autorepression property is lost.

These results suggest that the autorepression of the LFB1/HNF1 promoter and the *trans*-activation function map to different regions of the LFB1/HNF1 molecule.

DISCUSSION

It has been recently suggested that LFB1/HNF1 might play an important role in regulating the transcription driven by its own promoter. However, the results reported by different authors appear to be controversial as to whether LFB1/HNF1 behaves as an activator or a repressor of the transcription of its own gene (24–26).

In this paper, we present results indicating that the transcription of a reporter gene under the control of the LFB1/HNF1 promoter is in fact subjected to negative regulation by the LFB1/HNF1 protein, when transiently expressed in cultured hepatoma cells. This finding is in contradiction with the results reported by N.Miura and K.Tanaka (25), who propose that LFB1/HNF1 may bind to the proximal region of the promoter of its gene and transactivate its transcription in a synergistically fashion with HNF4. Zapp *et al.* (24) also observe a direct involvement of LFB1/HNF1 in the activation of transcription from the *Xenopus laevis* LFB1/HNF1 gene promoter. Our results are more consistent with the report of Kritis *et al.* (26) who observe an indirect negative autoregulatory loop responsible for the down-regulation of LFB1/HNF1 gene transcription. In line with the latter report, we could not identify any functional binding site

Figure 5. A region different from the activation domains is required for autorepression. C-terminal deletions of the LFB1/HNF1 protein were transfected in HepG2 cells with 10 μ g of the pE8U-3.5CAT reporter plasmid. Cells were harvested 36 h after transfection and the -3.5 kb promoter efficiency was determined from the CAT activity in whole-cell extracts. The results obtained by cotransfecting 1 μ g of the expression construct are reported. The values are expressed as the amount of CAT conversion corrected by the luciferase activity in the same cell extract (arbitrary units). The data represent an average of at least 2 independent experiments. A schematic representation of the LFB1/HNF1 protein is shown: open boxes: dimerization domain, pseudo POU-A and homeodomain; hatched boxes: Activation Domain I (aa 547–628) and III (aa 440–506).

for LFB1/HNF1 on its 3.5 kb promoter region: we scanned the entire -3.5 kb promoter in search of protein/DNA interactions by DNaseI footprinting and gel-shift experiments with a set of overlapping DNA fragments using purified LFB1/HNF1 protein. We could observe only a weak binding site centered at position -2677 from the transcriptional start site. However, this site has no involvement in the LFB1/HNF1 self-inhibition, since its deletion in the pE8U-2.6CAT construct affects neither the efficiency of transcription nor the autorepression (data not shown). In addition computer assisted analysis of the -3.5 kb LFB1/HNF1 promoter sequence has not revealed the presence of canonical LFB1/HNF1 DNA binding sites.

We have attempted to rule out relatively trivial explanations for this finding, such as competition for a limiting amount of a common target that is required for activation. Competition for a general transcription factor, a phenomenon known as 'squenching', seems unlikely on the basis of the following observations: in the same experimental system and at the same relative concentrations, LFB1/HNF1 is a potent *trans*-activator of liver-specific promoters and does not inhibit promoters that do not contain LFB1/HNF1 *cis*-acting elements (i.e. the RSV promoter). In addition, a construct carrying the activation domains of LFB1/HNF1 fused to a heterologous DNA-binding domain would be sufficient to mediate squenching, whereas the GAL4/LFB1 fusion protein did not have any effect on the activity of the LFB1/HNF1 promoter. We can also rule out the possibility that the over-expressed LFB1/HNF1 is sequestering a related transcriptional activator or an essential cofactor, such as DCoH, via its dimerization domain, since a mutant protein in which this region has been substituted by the TAT dimerization domain is still capable of repression. Moreover, it is unlikely that LFB1/HNF1 acts as a repressor of its own gene expression simply by displacing a more potent activator of transcription from its own promoter, because a truncated LFB1/HNF1 molecule

carrying only the DNA-binding domain, while able to repress the transcription from LFB1/HNF1-dependent promoters (31), was inactive on the LFB1/HNF1 promoter itself.

In addition to the DNA-binding domain we show that a sequence present in the carboxy-terminal region is also necessary for the autorepression effect. This region, tentatively located between aa 380 and 440, coincides with neither of the previously characterized liver-specific activation domains. One attractive possibility is that binding of LFB1/HNF1 to its own promoter occurs only if stabilized by protein-protein interactions, mediated by this latter region, with a second, unknown transcription activator: this protein-protein interaction would ultimately result in 'quenching' (40) the transcriptional potential of the second activator.

Autoregulation appears to be a common feature of genes coding for eukaryotic transcription factors: both positive and negative autoregulatory loops have been found to play an important role in modulating transcription factor expression (reviewed in 41 and 42). Many examples are known of positive and negative autoregulation of transcription factors: the muscle-specific factor MyoD1 activates its own endogenous gene when transfected into fibroblast cells inducing their conversion to myoblasts (43); the erythroid-specific factor GATA-1 (23) and the pituitary-specific POU-protein Pit1/GHF1 (22,44) have both been shown to positively regulate expression from their own promoter. Moreover, c-Fos generally represses its own transcription (21).

Several reports have contributed to outline a network of regulatory interactions among liver-specific transcription factors (19,20). Transcription of the LFB1/HNF1 gene depends on the presence of an active HNF4, thus indicating that, in the hierarchy of developmental regulation, HNF4 comes before the LFB1/HNF1 factor. In this paper we present evidence suggesting that another level of possible regulation of the transcription of the LFB1/HNF1 gene might be mediated by LFB1/HNF1 itself. It is worth noting that also LFB3/vHNF1 is capable of exerting a negative effect on LFB1/HNF1 transcription in HepG2 cells (21 and G.P., unpublished observations). In previous work we have shown that LFB3/vHNF1 mRNA, and the protein itself, is detected before LFB1/HNF1 during the early stages of development and during organ-specific differentiation (5,14). It could be speculated that the autoregulation of LFB1/HNF1 and the negative effect of LFB3/vHNF1 on LFB1/HNF1 transcription might play a role in guaranteeing the precise timing of the sequential activation of these two factors and their correct relative abundance, during critical stages of development and differentiation.

Further work will be required to elucidate the detailed mechanism by which this activity is brought about and to assess its biological relevance. At this stage, this phenomenon is just another tessera to be added to the complex mosaic of interdependent regulatory mechanisms potentially involved in liver-specific gene expression.

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