Vol. 196, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1993 Pages 480-486

# INTERPLAY OF THE LIVER-ENRICHED *TRANS*-ACTING FACTORS, DBP AND HNF1, IN THE TRANSACTIVATION OF HUMAN IGFBP-1 PROMOTER[+]

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Received August 23, 1993

**ABSTRACT** In the liver, expression of insulin-like growth factor binding protein-1 (IGFBP-1) is regulated essentially at the transcriptional level, at least in part by HNF1. In this study, the functional role of DBP and C/EBP (which have several potential binding sites on the IGFBP-1 proximal promoter) have been investigated. Transient co-transfection of the reporter plasmid, pBP-$1_{341}$ and eukaryotic expression vectors which code for DBP and C/EBP in human cell lines Hep3B, HepG2 and C33 showed that IGFBP-1 promoter activity was unchanged by C/EBP, but increased between 2 and 7 times by DBP (depending on the cell line). In addition, DBP and HNF1 were capable of functional co-operation in activating the IGFBP-1 promoter. Our results support the notion of DBP being involved in limited tissue specificity of IGFBP-1 expression. 

In biological fluids, insulin-like growth factors (IGFs) are non-covalently bound to high-affinity binding proteins (IGFBPs), of which there are at least six distinct species. These IGFBPs control the bioavailability of the IGFs and modulate their activity at the target cell level (1-3).

For each IGFBP species, tissue-distribution and hormonal and developmental regulation of expression are characteristic (3). In the case of IGFBP-1, high level of gene expression is detectable in the decidual cells of

[+]This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Association de la Recherche sur le Cancer (ARC) and the Ligue Nationale contre le Cancer.
S. Babajko is a recipient of a fellowship from the Ministère de la Recherche et de la Technologie.

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**ABBREVIATIONS:**
C/EBP: CCAAT/Enhancer Binding Protein, DBP: D site Binding Protein, HNF1: Hepatic Nuclear Factor 1, IGFBP-1: Insulin-like Growth Factor Binding Protein-1, CAT: Chloramphenicol Acetyl Transferase.

0006-291X/93 $4.00

the endometrium (4), in the liver during the neonatal period (5, 6) and regeneration (7) and in the hepatoma cell lines, Hep3B, HepG2, $H_{4\text{-}II}$ and $C_2$Rev7 (8, 9, 10).

IGFBP-1 gene expression in the liver is primarily regulated at the transcriptional level (6, 7). In order to understand the mechanisms of this control, it is necessary to characterize the *trans*-acting factors involved. To date, the only *trans*-activator shown to be functionally active is HNF1 (8, 9).
A computer search for homology identified *cis*-acting sequences within the first 340 base pairs 5-' of the transcription initiation site that are homologous to the consensus sequences of liver-enriched *trans*-acting factor binding sites for C/EBP, DBP, HNF3 and HNF4 (11). In rat liver, C/EBP expression is maximal during the perinatal period (12), like that of IGFBP-1 (5, 6). DBP (13) and IGFBP-1 (14) both exhibit circadian rhythms in their expression. In view of these similarities C/EBP and DBP were given priority in our studies of the control of IGFBP-1 promoter transcriptional activity.

Their functional roles and possible co-operation with HNF1 were therefore investigated using transient co-transfection in various cell lines and eukaryotic expression vectors coding either for the *trans*-activators or for the CAT reporter gene placed under IGFBP-1 promoter control.

## MATERIALS AND METHODS

**Cell culture.**
Human cervical carcinoma cells ($C_{33}$) and the human hepatoma cell line, HepG2 were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. The human cell line Hep3B, was cultured in $\alpha$-medium supplemented with 10% fetal calf serum and antibiotics.

**Plasmids.**
pBP-$1_{341}$ and pBP-$1_{341rev}$ are eukaryotic expression vectors in which CAT gene expression is driven by the human IGFBP-1 promoter (nt -341 to +1) inserted in correct or reverse orientation. In pBP-$1_{mut60/72}$, the IGFBP-1 promoter was mutated at the HNF1 binding site (8).
pUTKAT$_4$ contains the thymidine kinase promoter fused with the CAT gene (15). pCMV-DBP (16), pMSV-C/EBP (17), pRSV-HNF1 (18) are eukaryotic expression vectors that generate high levels of DBP, C/EBP and HNF1 expression in transfected cells, respectively. pCH110 was obtained from Pharmacia (Sweden).

**Transient transfection.**
Cells were seeded at a density of $2x10^6$ cells per 10 cm Petri dish and transfected using the previously described calcium/phosphate co-precipitation procedure (8). Transfection efficiency was monitored by co-transfection of 3 $\mu$g of pCH110 and quantification of $\beta$-galactosidase activity in all extracts (19).

**Isolation of RNA and Northern blot analysis**
Total RNA was extracted from monolayers of cultured cells and separated by agarose gel electrophoresis and blotted onto nylon membranes (8). Northern

blots were hybridized as previously described (8), with IGFBP-1 cDNA, DBP cDNA and α-tubulin cDNA probes.

## RESULTS AND DISCUSSION

### Functional role of C/EBP in IGFBP-1 promoter activity

In order to determine whether or not C/EBP is functionally involved in IGFBP-1 proximal promoter activity, two human hepatoma cell lines, Hep3B and HepG2, and a human cervical carcinoma cell line, $C_{33}$, which fails to express liver-enriched *trans*-acting factors, were co-transfected with pBP-$1_{341}$ either alone or in the presence of pMSV-C/EBP.

With 5 μg pMSV-C/EBP, IGFBP-1 promoter activity in Hep3B cells was 0.9 ± 0.3 times that of the promoter transfected alone, in HepG2 cells, 1.2 ± 0.3 times, and 1.2 ± 0.2 times in $C_{33}$ cells (where activation of IGFBP-1 promoter by HNF1 (5.9 times) remained unchanged in the presence and absence of C/EBP) (n=4, mean ± SEM) (Figure 1). This lack of stimulation by C/EBP was observed whatever the quantity of pMSV-C/EBP used (1 to 10 μg). Since the absence of effect could be due either to C/EBP's not *trans*-activating the IGFBP-1 promoter or to some artefact, controls were run to check that under our experimental conditions C/EBP was active on the thymidine kinase promoter which is known to be *trans*-activated by it (20). Co-transfection of 10 μg pUTKAT$_4$ and 5 μg pMSV-C/EBP in Hep3B cells led to a 3-fold increase over the thymidine kinase promoter activity obtained with pUTKAT$_4$ alone, thereby confirming that our transfected C/EBP was indeed functional (Figure 1).

From these results it can be concluded that C/EBP plays no significant role in IGFBP-1 proximal promoter activity.

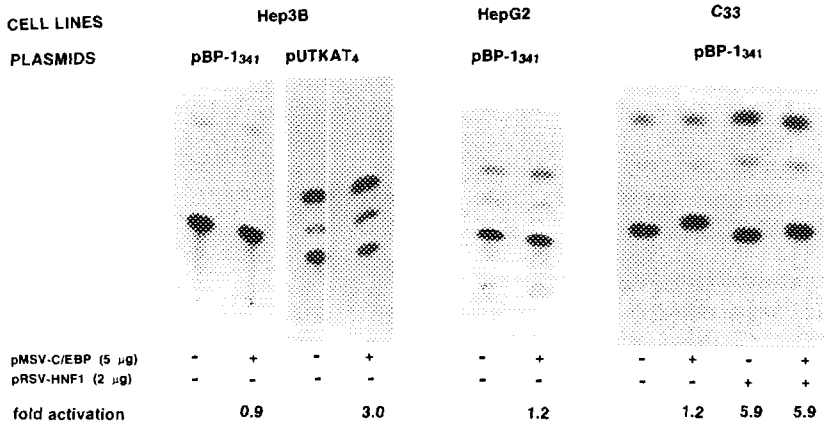


**Figure 1.** Functional role of C/EBP in IGFBP-1 promoter activity.
10 μg plasmid reporters (pBP-$1_{341}$ or pUTKAT$_4$) was transfected with or without 5 μg pMSV-C/EBP.

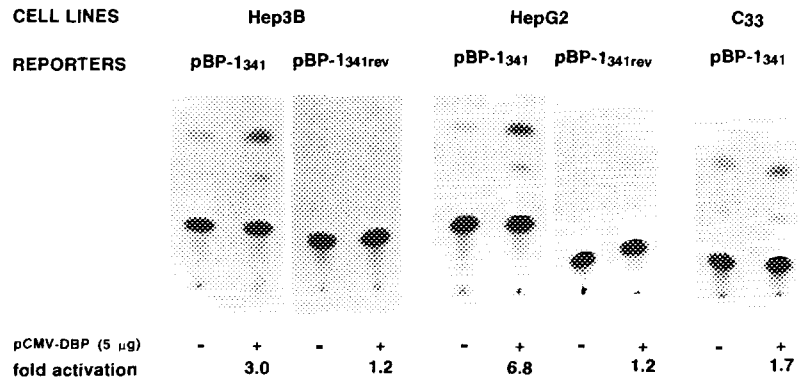


**Figure 2.** Functional role of DBP in IGFBP-1 promoter activity.
10 µg plasmid (pBP-$1_{341}$ or pBP-$1_{341rev}$) was transfected with or without 5 µg pCMV-DBP.

**Funtional role of DBP in IGFBP-1 promoter activity**

The same methodological approach was used to investigate the possible involvement of DBP in activating transcription from the IGFBP-1 promoter.

With co-transfection of 5 µg pCMV-DBP and pBP-$1_{341}$, IGFBP-1 promoter activity was increased 3.0 ± 0.6-fold in Hep3B cells, 6.8 ± 0.9-fold in HepG2 and 1.7 ± 0.6-fold in $C_{33}$ cells compared to that obtained when pBP-$1_{341}$ was transfected alone (n=3 or 4, mean ± SEM). No *trans*-activation of the IGFBP-1 promoter inserted in reverse orientation (pBP-$1_{341rev}$) was observed (Figure 2). The same results were obtained with 2 and 5 µg pCMV-DBP, which indicates that our experimental conditions were optimal for each cell line. DBP is therefore capable of activating transcription from the IGFBP-1 promoter in cultured cells, whatever their origin. Controls were run to check that, compared with that of the ubiquitous α-tubulin gene, expression of the endogenous IGFBP-1 gene was about 5 times greater in Hep3B cells transfected with 5 µg pCMV-DBP than in control transfected cells (Figure 3).
From all our results it would seem that DBP may play a role in controlling the IGFBP-1 expression. The functionality of the site(s) of the interaction between DBP and the IGFBP-1 promoter is currently under investagation in this laboratory

**Modulation of DBP's functional role by HNF1**

Since DBP activation of the IGFBP-1 promoter in $C_{33}$ cells was a quarter of that in HepG2 hepatoma cells and half of that in Hep3B cells (Figure 2, Table 1), it would seem either that DBP is less active in $C_{33}$ cells or that other *trans*-acting factors may co-operate with DBP in hepatoma cells, particularly those that are liver-enriched. HNF1, a liver-enriched *trans*-acting factor which is known to *trans*-activate the IGFBP-1 promoter (8), was tested for such

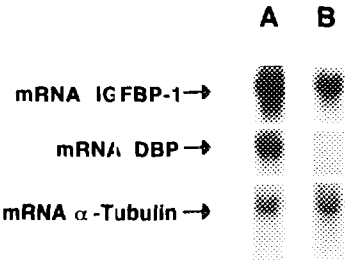


**Figure 3.** Functional role of DBF in endogenous IGFBP-1 gene expression. RNA of transfected cells (with (**A**) or without (**B**)) were hybridized to IGFBP-1, DBP and α-tubulin cDNA probes

functional co-operation by transient co-transfection experiments comparing the wild-type promoter (pBP-$1_{341}$) with an IGFBP-1 promoter mutated at the HNF1 *cis*-element (pBP-$1_{mut60/72}$), where no potential DBP recognition site was identified either within or near to the mutated sequences. This mutation totally abolishes HNF1 activation (8) and is therefore useful in studying DBP activation independently of any functional interaction with HNF1 in hepatoma cell lines. DBP activation of the mutated promoter was 1.9 fold in the Hep3B, and 4.8 fold in the HepG2 cells (n=2), which corresponds to 63% of activation of the wild-type promoter in both cell lines (Table 1). Co-operation between DBP and HNF1 was confirmed by co-transfection of pRSV-HNF1 and pCMV-DBP in $C_{33}$ cells (which do not express HNF1 (21)), where IGFBP-1 promoter activity was increased 10.4 ± 1.5 times (n=3), whereas with DBP alone the increase was only 1.7 ± 0.6 times (n=4) and with HNF1 alone, 5.1 ± 1.6 times (n=3, mean ± SEM) (Table 1). We also observed that DBP's functional role was not modulated by C/EBP (data not shown) although it has been reported either

**Table 1**: Modulation of DBP activity by HNF1

| | | PLASMIDS | | |
|---|---|---|---|---|
| CELL LINE | REPORTER | pCMV-DBP | pRSV-HNF1 | activation |
| Hep3B | pBP-$1_{341}$ | + | - | **3.0** ± 0.6 (n=3) |
| | pBP-$1_{mut60/72}$ | + | - | **1.9** (n=2) |
| HepG2 | pBP-$1_{341}$ | + | - | **6.8** ± 0.9 (n=3) |
| | pBP-$1_{mut60/72}$ | + | - | **4.8** (n=2) |
| C33 | pBP-$1_{341}$ | + | - | **1.7** ± 0.6 (n=4) |
| | pBP-$1_{341}$ | - | + | **5.1** ± 1.6 (n=3) |
| | pBP-$1_{341}$ | + | + | **10.4** ± 1.5 (n=3) |

Cells were transfected with 10 μg pBP-$1_{341}$ (wild-type) or pBP-$1_{mut60/72}$ (promoter mutated at HNF1 binding site) with or without pCMV-DBP and pRSV-HNF1. (Data represented mean ± SEM).

to compete for the same site (22) or potentiate its action (23). Our results together suggest (i) that HNF1 and DBP are involved in the transcriptional control of the IGFBP-1 gene, (ii) that DBP, which is only found in the liver (16) would be one of the factors involved in determining liver-specificity of IGFBP-1 gene expression and (iii) that more than one functional interactions (eg. HNF1 and DBP) occur *in vivo* to achieve optimal promoter activity.

Our results therefore agree with those obtained for other genes expressed in the liver (eg. albumin, phosphoenolpyvurate carboxykinase, aldolase B, $\alpha$-2,6 sialyltransferase) that highlight the multiplicity of interactions amongst promoters in determining the tissue-specificity of expression (11).

## ACKNOWLEDGMENTS

We thank Drs. U. Schibler, S.L. McKnight, M. Yaniv for providing DBP, C/EBP$\alpha$ and HNF1 expression vectors, respectively. We thank Dr. M. Binoux for fruitful discussions and Miss P. Leneuve for technical assistance.

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