Tissue-specificity of liver gene expression: a common liver-specific promoter element

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ABSTRACT

Several liver specific genes contain a sequence similar to the hepatocyte-specific promoter element (HPI) identified in the *Xenopus* albumin gene. By competition experiments in gel retardation assays we demonstrate that the albumin, the α_1 -antitrypsin and the β -fibrinogen promoter elements bind an identical factor, whereas the HPI-related sequence in the transferrin gene has different binding properties. The elements of all four genes are able to confer increased transcription in nuclear extracts from rat liver. The HPI found in the albumin, α_1 -antitrypsin and β -fibrinogen promoter bind identical transcription factors, since in vitro transcription can be specifically inhibited by the addition of an excess of the corresponding oligonucleotide. The cis-element HPI and its transacting factor have been conserved during evolution, since elements derived from frog, rat and human genes function in rat liver nuclear extracts.

INTRODUCTION

A large part of tissue-specific protein synthesis in higher eukaryotes is achieved by selective transcription of a limited set of genes. Gene transfer experiments have revealed tissue-specific enhancer and promoter elements in many genes (1). One would speculate that different genes active in a given tissue contain common regulatory elements that allow coordinate expression of various genes using the same trans-acting factor. Liver cells express a series of well characterized genes e.g. albumin (2,3), α_1 -antitrypsin (4,5), β -fibrinogen (6), and transferrin (7), which are predominantly active in hepatocytes and are therefore a good system to search for regulatory elements common for different genes. We have recently identified in the *Xenopus* albumin gene (8) a hepatocyte-specific promoter element (HPI) which has some similarity with promoter elements identified in the human α_1 -antitrypsin (4) and the rat β -fibrinogen gene (6). In this report we demonstrate by competition experiments using a binding assay as well as in vitro transcription, that these 3 different genes contain a common liver-specific promoter element.

MATERIAL AND METHODS

Plasmid constructions

Syn 0-TG was constructed as described previously (8) and the oligonucleotides AT, FB, TF (see Fig.1) were used to replace syn 0 in the transcription vector syn 0-TG. As a negative control (PL) we replaced syn 0 by the polylinker sequence (HindIII-BglII fragment) of pBL CAT3 (13). As a positive control (AML) we used clone pML (C₂AT)₁₉ as described by Sawadogo and Roeder (9). As an internal control we used a derivative of clone pML (C₂AT)₁₉ i.e. pML cas 9, with a shortened -G- cassette of 190 nt.

Band shift assays

Nuclear extracts were essentially prepared from rat liver as described by Gorski et al. (10). 5 µg nuclear proteins were incubated in 20 µl binding buffer (11) containing 250 ng of unspecific competitor poly (dI-dC) · poly (dI-dC) and 10⁴ cpm of ³²P-labelled oligonucleotides as given in the figure legend. After 30 min incubation at room temperature, the resulting complexes were resolved on a 4% polyacrylamide gel as described by Sing et al., (11). The gel was fixed in 10% acetic acid, dried and exposed for autoradiography.

Preparation of labelled oligonucleotides

The oligonucleotides were synthesized with the Pharmacia Gene Assembler and purified on a preparative 20% polyacrylamide gel in 8M urea as described (8).

In vitro transcription assays

The in vitro transcription was done as described (8) with 7 µl nuclear extract in a total volume of 20 µl, using 35 ng of the corresponding transcription vector and as internal control 17 ng pML cas 9. For competition experiments 100 ng of the indicated oligonucleotide (see Fig.3c) was included in the reaction. The transcription products were separated on a sequencing gel and exposed for autoradiography (3 b, 40 hrs; 3 c, 16 hrs). The transcription activity of the various constructs was determined by densitometry of the bands using the pML cas 9 signal as reference. The values were standardized to the activity found without added competitor.

RESULTS

The promoter region of the albumin, α-fetoprotein α₁-antitrypsin, A-fibrinogen and transferrin gene contains a similar sequence element

Transfection of various Xenopus albumin-CAT genes into mammalian cells allowed us to identify the promoter element HP1 which confers selective expression in hepatoma cells and mediates high in vitro transcription in rat

Figure 1: Sequences of the promoter elements used for binding assay and in vitro transcription

The hepatocyte-specific promoter element (HP1) of the *Xenopus* 68 kd albumin gene (12) is given at the top as it is present in the synthetic oligonucleotide syn 0. The boxed area is the functional element we have defined and the three positions of point mutations that destroy the function are indicated by a dot (8). The oligonucleotides corresponding to regulatory elements as found in the genes coding for human α_1 -antitrypsin (AT), human α_1 -antitrypsin mutant (AT-mt), rat β -fibrinogen (FB), human transferrin (TF), mouse albumin (Alb) and mouse α -fetoprotein (AFP) are aligned in such a way to attain maximal homology to HP1 in syn 0. The position relative to the cap site is given and the HindIII and BglII site used for cloning are included.

liver nuclear extracts (8). This hepatocyte-specific promoter element is defined as a 13 bp sequence (syn 0 in Fig.1), which is recognized by a hepatocyte-specific transcription factor (8). The fact that this regulatory element is derived from a frog gene and functions in mammalian cells demonstrates that it has been conserved during evolution.

This interpretation is supported by our observation that sequences similar to HP1 are also found in the promoter of the mammalian albumin and α -fetoprotein (AFP) genes (8). The sequences as found in the mouse genome are listed at the bottom of Fig.1

Figure 2: Promoter elements of the albumin, α_1 -antitrypsin and the β -fibrinogen gene bind the same factor

In lane 1 nuclear proteins prepared from rat liver were incubated with the following ^{32}P -labelled oligonucleotides: a) syn 0, b) AT, c) FB and d) TF (see Fig.1 for abbreviations used). Lanes 2 to 8 additionally contain 6 ng of the indicated unlabelled oligonucleotide as competitor. The samples were analyzed on a 4% polyacrylamide-gel (11).

Several experiments performed in other laboratories suggested to us that elements similar to HP1 are also present in other liver-specific genes: Cortese's group (4) demonstrated by transfection of human α_1 -antitrypsin gene constructs several elements involved in tissue-specific gene

expression. A linker scanning mutant (pml in ref.4) destroys a promoter proximal element that is similar to HP1 (Fig.1). Furthermore Courtois et al. (6) recently reported a common liver-specific nuclear factor which interacts with both the β -fibrinogen and the α_1 -antitrypsin gene promoter (6). The corresponding sequences are listed in Figure 1. In addition, the analysis of the human transferrin gene (personal communication by F. Brunel, E. Schaeffer and M.M. Zakin, for sequence see ref. 7) identified a sequence similar to HP1 (see Fig.1).

Several liver-specific genes contain the promoter element HP1 which interacts with the same proteins

To investigate whether similar sequence elements found in various genes represent an identical regulatory element we have synthesized the corresponding oligonucleotides given in Fig.1 and compared their binding properties to proteins of rat liver nuclear extracts. Figure 2 illustrates gel retardation assays using essentially the same conditions as previously described to define the hepatocyte-specific interaction of syn 0, the HP1 element present in the Xenopus albumin gene (8). A similar prominent complex of low mobility as with syn 0 is observed with the α_1 -antitrypsin and the β -fibrinogen oligonucleotide (compare lane 1 in panels a, b and c) whereas with the transferrin oligonucleotide no distinct complex formation is detected, but rather a smear along the entire track (lane 1, panel d). To reveal whether the complexes formed with the different oligonucleotides contain the same proteins, we carried out competition experiments by adding an excess of various unlabelled oligonucleotides. Clearly, the oligonucleotides as found in the albumin, α_1 -antitrypsin and β -fibrinogen elements bind the same factor whereas the transferrin element binds different proteins. In addition we also used the oligonucleotide containing the α_1 -antitrypsin mutant (ATmt, in Fig.1) which competes only weakly. This low competition correlates with the observation that the labelled ATmt probe gives no complex formation under the binding conditions used (data not shown). However, oligonucleotides corresponding to the HP1 homologues as found in the mouse albumin and α -fetoprotein (AFP) genes (see Fig.1 for sequence) compete as efficiently as syn 0. The fact that the smear observed with the labelled transferrin sequence is only competed by the transferrin oligonucleotide itself, further illustrates that the transferrin element is distinct. Using the α_1 -antitrypsin oligonucleotide, a faster migrating complex was also observed, which can only be competed by the unlabelled α_1 -antitrypsin and AFP oligonucleotides. We speculate that this faster

Figure 3: Various promoter elements are transcriptionally active in rat liver nuclear extracts

a) Schematic drawing of syn 0-TG (not to scale), used for in vitro transcription. The plasmid is based on pBL CAT3 (13) and contains HP1 as well as the TATA region of the *Xenopus* albumin gene (-67 to -26) linked to the -G-cassette (8).

b) In vitro transcription products were analyzed on a sequencing gel. The various constructs included in the reactions are indicated above the lanes (AT, FB, TF were used to replace syn 0 in syn 0-TG, see Fig.1 for abbreviations used). Additionally we used as a negative control a polylinker sequence (PL) and as a positive control the adeno major late promoter (AML). HaeIII digested pBR322 is used as size marker (m). Signals of the transcription vector and of the internal control are marked by exp. and contr., respectively.

c) As competitor 100 ng of the oligonucleotide FB or TF (see Fig.1) is added in the reactions as indicated.

migrating complex represents a distinct protein-DNA interaction. In summary, the binding assays establish that the elements found in the albumin, AFP, α_1 -antitrypsin and β -fibrinogen gene bind the same factor. We therefore consider all these elements as HP1 homologues. The identity of the binding at the α_1 -antitrypsin and β -fibrinogen binding site confirms earlier data (6).

The promoter element HP1 of the albumin, α_1 -antitrypsin and β -fibrinogen gene interacts with the same transcription factor

Our previous experiments have demonstrated that HP1 as found in the *Xenopus* albumin gene, i.e. syn 0, is able to stimulate in vitro transcription in rat liver nuclear extracts (8). To analyze whether the HP1 homologues found in the other genes can also increase the transcriptional activity, we replaced the *Xenopus* HP1 sequence in syn 0-TG (see Fig.3a) with these homologues. The various constructs were tested in the in vitro transcription system using

Figure 4: The promoter elements of the albumin, α_1 -antitrypsin and β -fibrinogen interact with the same transcription factors

In vitro transcriptions as shown in Fig.3 were performed using the various oligonucleotides listed in Fig.1 as competitor (100 ng per reaction). In a, b and c the transcription vectors containing HP1 of the *Xenopus* albumin (syn 0-TG) the human α_1 -antitrypsin (AT-TG) and the β -fibrinogen (FB-TG) genes were used, respectively. The data were quantified by densitometry of the autoradiograms using the internal control pML cas 9 for reference. The values were standardized to the activity found without (-) added competitor.

the -G-cassette of about 400 bp (9). In all assays the adeno major late promoter with a shortened -G-cassette of 200 bp was used as an internal standard to quantitate the in vitro transcripts. Figure 3b demonstrates that all four oligonucleotides confer a positive transcriptional activation to the TATA box in the rat liver nuclear extract. The β -fibrinogen element (FB) is as active as the *Xenopus* albumin element (syn 0), whereas the α_1 -antitrypsin (AT) and transferrin (TF) element give approximately 3-fold lower activity which is still 5-10 fold higher than the activity observed with a control construct containing no regulatory sequence (PL). We have previously shown that transcription of the syn 0-TG construct containing the *Xenopus* albumin HP1 can be specifically inhibited by an excess of added

oligonucleotide syn 0 (8). This observation allows us to compare the regulatory elements by the addition of various oligonucleotides as competitor. Fig.3c illustrates that the addition of the β -fibrinogen oligonucleotide (FB) inhibits the transcription of the β -fibrinogen containing transcription vector as well as the activity of syn 0-TG which contains HP1 of the *Xenopus* albumin gene. This is a specific effect as the adeno major late promoter is not influenced. Thus, a common transcription factor recognizes both these elements. However, the transferrin oligonucleotide (TF) does not interfere with the activity of the transcription vectors containing the *Xenopus* albumin or the β -fibrinogen HP1. A series of similar competition assays is summarized in Fig.4. The quantification of the data (panel a) shows that the activity of syn 0-TG containing the *Xenopus* albumin HP1 is inhibited by at least 90% on addition of the HP1 containing oligonucleotide of the α_1 -antitrypsin and the β -fibrinogen gene as well as the HP1 homologues found in the mouse albumin and α -fetoprotein gene. No clear competition is observed with the α_1 -antitrypsin mutant and the transferrin oligonucleotides. Panels b and c summarize complementary experiments using the transcription vector containing either the β -fibrinogen or α_1 -antitrypsin element. In both cases the oligonucleotides containing the HP1 of the *Xenopus* albumin, the α_1 -antitrypsin or the β -fibrinogen show efficient inhibition, whereas the transferrin oligonucleotide is a weak competitor.

DISCUSSION

Taken together our functional competition assays confirm the binding studies, that the albumin, α_1 -antitrypsin and β -fibrinogen oligonucleotide bind identical proteins. Clearly, the albumin, α_1 -antitrypsin and the β -fibrinogen gene contain a common promoter element HP1, recognized by the same transcription factor and this cis-element is distinct from that found in the transferrin gene. We speculate that the regulatory sequence in the transferrin gene represents another hepatocyte-specific promoter element.

We have previously observed by the introduction of point mutations into HP1 of the *Xenopus* albumin gene that the first, third and 13th position of the element is crucial for its function in vivo (8). Based on this definition the β -fibrinogen element would not be functional as it is mutated at the third position compared to the *Xenopus* albumin element. On the other hand the transferrin element would fit to the albumin HP1. Obviously we are as yet unable to predict all the sequences that may form a functional HP1.

However, using a competition assay in the in vitro transcription, unambiguous identification is possible.

Based on the present finding that HP1 is also present in the β -fibrinogen gene, it is most likely that the hepatocyte nuclear factor 1 (HNF1) identified as a binding protein in the β -fibrinogen promoter region -102 to -75 (6) is identical to the factor we have identified in this paper. However, we are not sure whether α 1TF-B described by Cortese's group (4) as component binding to the region homologous to HP1 in the α_1 -antitrypsin gene, represents the same component, since our gel retardation experiments always reveal two complexes with the α_1 -antitrypsin sequence (see Fig.2b) and only the slower migrating form can be competed with the other HP1 elements. In the case of the mouse albumin gene several DNA-binding proteins have been found to bind to the promoter (2,3). From these footprint studies we assume that the factor binding to site B (2) and to the proximal element which contains HP1 (3), is identical to the transcription factor recognizing HP1 in our experiments.

In conclusion our data confirm for the first time that 3 different genes specifically expressed in the same tissue contain a common tissue-specific promoter element which is recognized by the same transcription factor. Clearly, the cis-element HP1 and the corresponding trans-acting factor have been conserved during evolution, since elements derived from frog, rat and human genes function in rat liver nuclear extracts. The simplest interpretation we would provide to these data is the assumption that a single protein is involved in the interaction with HP1 and that the complex as seen in the gel retardation assay leads to an increase in tissue-specific transcription. Our previous experiments established that HP1 is recognized by a nuclear protein specific for hepatic cells (8). This suggests that the gene coding for this protein controls the expression of several liver specific-genes. It will be of interest to determine at which stage of embryogenesis and organogenesis this gene is activated.

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