

Transcriptional activation function of hepatitis B virus Pre S1 protein in yeast

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OBJECTIVE: To explore the feasibility of cloning of the hepatocyte receptor interacting with the Pre S1 protein of HBV by two-hybrid system.

METHODS: Yeast expression plasmids encoding fusion proteins of full length or portions of Pre S1 of HBV and DNA binding domain of yeast protein GAL4 were constructed and used to transform yeast reporter strain SFY526. Reporter gene product β -galactosidase activity was assayed as a measure of transcriptional activation in yeast. Mammalian expression plasmid encoding fusion proteins of full length Pre S1 and DNA binding domain of GAL4 was constructed and used to cotransfect hepatoma cell line Huh-7 together with CAT reporter plasmid. Cell extracts were assayed for CAT activity by thin-layer chromatography.

RESULTS: The fusion proteins of full length Pre S1 protein and GAL4 DNA binding domain presented transcriptional activation function in yeast. The transcription activating sequence was localized to the 21 to 47 amino acids of Pre S1 protein. Fusion proteins of full length Pre S1 and GAL4 DNA binding domain did not show transcriptional activation function in mammalian cells.

CONCLUSIONS: The transcription activating sequence of HBV Pre S1 protein in yeast overlaps the hepatocyte receptor binding site. The transcriptional activation function of HBV Pre S1 protein in yeast may prevent researchers from using yeast two-hybrid system to clone HBV receptor interacting with Pre S1 protein. However, the Pre S1 protein does not show transcriptional activation function in mammalian cells. Mammalian two-hybrid system may be a practical method to clone the HBV hepatocyte receptor interacting with Pre S1 protein.

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Key words: HBV; Pre S1 protein; transcriptional activation; yeast

Introduction

Experimental evidence indicates that Pre S1 protein of HBV participates in hepatocyte re-

ceptor binding. Antibodies to Pre S1 protein block the binding of HBV virions to liver plasma membranes and to human hepatoma cell line (HepG2). A Pre S1 sequence (21–47 amino acids) is demonstrated to be directly involved in hepatocyte receptor binding.^[1] However, the receptor for HBV remains elusive. Although numerous candidates have been identified and characterized in greater or less detail, none has fulfilled all of the criteria necessary for its identification as the HBV receptor.^[2] Identification and cloning of the receptor for HBV would greatly facilitate research into the pathogenesis and drug design of hepatitis B. Two-hybrid system as a genetic assay to detect protein-protein interactions in vivo provides a method to

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identify and clone genes for proteins that interact with a target protein.^[3,4] So it is also an efficient system to study interactions between viruses and cellular proteins and to clone unknown receptors. Before being used in hepatocyte cDNA library screening to clone HBV hepatocyte receptor, the Pre S1 protein (target protein) should be tested for transcriptional activation function in an appropriate reporter strain.^[5] In this study we found that HBV Pre S1 protein has transcriptional activation function in yeast by constructing fusion proteins of Pre S1 and DNA binding domain of yeast protein GAL4 and that the transcriptional activating sequence of Pre S1 protein is localized in yeast.

Methods

Plasmids

PGBT9, a 5.4-kb DNA binding domain hybrid cloning vector, is used to generate fusions of Pre S1 with GAL4 DNA binding domain and has the transformation marker tryptophan (Trp). PGAD 424 is a 6.6-kb transcription activation domain hybrid cloning vector encoding the GAL4 transcription activation domain and leucine (Leu). PCL1 is a 15.3-kb positive control plasmid encoding and expressing the full-length, wild type GAL4 protein and Leu.

Yeast reporter strains

SFY526 has a Lac Z reporter gene under control of GAL4 upstream activating sequence; HF 7C has two reporter genes; a Lac Z gene and a HIS3 gene under the control of GAL4-responsive sequences. Both SFY526 and HF 7C have the transformation markers; Trp and Leu, i.e.; these yeast strains can not grow in synthetic medium lacking Trp and Leu, unless they are transformed by a plasmid carrying the wild-type version of these genes (Trp1, Leu2). All the above plasmids and yeast reporter strains are the components of Matchmaker two-hybrid system (Clontech, USA).

Construction of yeast expression plasmids encoding fusion proteins of full length or portions of HBV Pre S1 and DNA binding do-

main of GAL4

The Pre S1 fragments were generated by PCR with restriction site EcoR I on 5' end and Sal I on 3' end, and inserted into plasmid PGBT9. The sequences and reading frame of fusion gene were verified by sequencing.

Construction of mammalian expression plasmid encoding fusion proteins of full length of HBV Pre S1 and DNA binding domain of GAL4

Full length Pre S1 sequence was cloned to Sal I, Spe I sites of plasmid PPC62^[6] and then the fragment between Hind III and Not I of the PPC62 which contains the fusion gene of GAL4 DNA binding domain and the Pre S1 sequence was inserted into the two Not I sites of mammalian expression vector PCMVβ (Clontech, USA). The resulting construction was designated as PCMVSI which encodes fusion of the GAL4 DNA binding domain and the Pre S1 protein instead of original reporter gene product β-galactosidase. The orientation and reading frame of the fusion gene were checked by restriction enzyme digestion and sequencing.

Transformation of yeast strains

Yeast reporter strains were transformed by the lithium acetate method.^[7] The transformants were plated to the appropriate selection medium and incubated at 30 °C for 2–4 days until colonies appear.

β-galactosidase assays

Yeast colonies were assayed for β-galactosidase activity by filter-transferring the colonies to SSX plates and incubated at 37 °C.^[4] The SSX plates contained 6.7 g yeast nitrogen base without amino acids, 14 g agar, 75 mg X-gal, 100 ml 1 mol potassium phosphate (pH 7.0), 100 ml 20% (wt/v) sucrose, 100 ml 10 × amino acids minus Trp or Trp and Leu, and H₂O to 1 liter.

DNA transfection and CAT assay

Hepatoma cell line Huh-7 was transfected by the calcium phosphate method with 5 μg each CAT

reporter plasmid, PG6 (-119) HIV LTR Δ TAR with 6 GAL4 binding sites^[8] or PHIV-1 CAT (-121/+232) without GAL4 binding site,^[9] and plasmid PCMV S1 which encodes fusion of GAL4 DNA binding domain and Pre S1 protein or plasmid pCMV β as control. After 2 days, cell extracts were assayed for CAT activity by thin-layer chromatography.^[10]

DNA sequencing

DNA sequencing was performed by the dideoxynucleotide chain termination method (DNA sequence kit, USB Company, USA).

Results

Fusion proteins of full length or portions of HBV Pre S1 and DNA binding domain of GAL4 present transcriptional activation function in yeast

Yeast expression plasmid encoding fusion proteins of full length Pre S1 and DNA binding domain of GAL4 (PGBT9-S1) was used to transform yeast reporter strain SFY526. The transformants were plated to the selection medium minus tryptophan and incubated at 30 °C until the size of colonies reached 1–2 mm in diameter. The colonies were transferred to SSX plates and incubated at 37 °C.

After 3 hours, the colonies turned blue. The plasmid PGBT9 and PCL1 were used as negative and positive controls, and the expected results were obtained. These results indicate that the fusion proteins of full length Pre S1 and DNA binding domain of GAL4 could activate transcription of reporter gene Lac Z in yeast.

To localize the transcription activating sequence of the Pre S1 protein, which consists of 108–119 amino acids (AA), 3 plasmids encoding fusions of GAL4 DNA binding domain and portions of Pre S1 protein, PGBT9-S1 (12-91AA), PGBT9-S1 (71-119AA), PGBT9-S1 (21-47AA) were constructed because HBV Pre S1 protein consists of 108–119 amino acids (AA). By transforming the yeast reporter strain SFY526 and detecting β -galactosidase activity of transformant colonies, fusion proteins of Pre S1 (21-47AA) and GAL4 DNA binding domain could activate transcription, fusion of Pre S1 (12-91AA) and GAL4 DNA binding domain activated transcription when the yeast reporter strain was cotransformed by plasmid PGAD424 expressing the GAL4 transcriptional activation domain and fusion of Pre S1 (71-119AA) and GAL4 DNA binding domain did not show transcriptional activation function (Table 1). So, the transcriptional activating sequence of Pre S1 protein was localized to 21–47 amino acids which is also the

Table. Transcriptional activation by Pre S1 protein in yeast

Transformants		Selection medium	β -gal assay (colony color)	Transcription activation
Plasmid 1 (GAL4 DNA binding domain or fusions)	Plasmid 2 (GAL4 DNA activation domain)			
PGBT9	—	—Trp *	white	—**
PCL1	—	—Leu +	blue	+***
—	PGAD424	—Leu	white	—
PGBT9	PGAD424	—Trp, —Leu ++	white	—
PGBT9-S1 (full length)	—	—Trp	blue	+
PGBT9-S1 (12-91AA)	—	—Trp	white	—
PGBT9-S1 (12-91AA)	PGAD424	—Trp, —Leu	blue	+
PGBT9-S1 (71-119AA)	—	—Trp	white	—
PGBT9-S1 (71-119AA)	PGAD424	—Trp, —Leu	white	—
PGBT9-S1 (21-47AA)	—	—Trp	blue	+

* Selection medium minus tryptophan; + selection medium minus leucine; ++ selection medium minus tryptophan and leucine; ** no transcription activation; *** transcription activation.

hepatocyte receptor binding site.

PCBT9-S1 (71-119AA) and human liver cDNA library (activation domain library) were co-transformed into the yeast reporter strain HF 7C. By screening, no positive clone was found. This result further suggests that the fragment between 71 to 119 amino acids of Pre S1 is not the hepatocyte receptor binding site.

Fusion proteins of full length of HBV Pre S1 and GAL4 DNA binding domain did not show transcriptional activation function in mammalian cells

To determine whether HBV Pre S1 protein could activate transcription in mammalian cells, we transfected plasmid PCMVSI encoding fusion of GAL4 DNA binding domain and Pre S1 protein or plasmid PCMV β as controls into hepatoma cell line Huh-7 together with each of the CAT reporter plasmids; PG6 (-119) HIV LTR Δ TAR or PHIV-1 CAT (-121/+232). The fusions of Pre S1 protein and GAL4 DNA binding domain were incapable of activating transcription of the CAT gene from the promoter carrying GAL4 binding sites.

Discussion

The two-hybrid assay is based on the fact that many eukaryotic transcriptional activators (including GAL4) consist of two physically separable modular domains: one acts as the DNA-binding domain, while the other functions as the transcriptional activation domain. The DNA-binding domain localizes the transcription factor to specific DNA sequences present in the upstream region of genes that are regulated by this factor, while the activation domain contacts other components of the transcription machinery required to initiate transcription. Both domains are required for normal activation functioning, and normally the two domains are part of the same protein. However, it has been shown that a functional activator can be assembled *in vivo* from separated domains of the same or unrelated transcription factors via recombinant DNA technology.^[3] In the Matchmaker two-hybrid system, sequences encoding the two functional do-

main of the GAL4 transcriptional activator have been cloned into two different shuttle/expression vectors. The PCBT9 hybrid cloning vector is used to generate a fusion of the GAL4 DNA-binding domain with a target protein (X). The PGAD424 hybrid cloning vector is used to generate a fusion of the GAL4 activation domain with a potentially interacting protein (Y) or with a collection of random proteins in a fusion library. If proteins X and Y interact with each other, the DNA-binding domain of GAL4 will be tethered to its transcriptional activation domain, and the proper function of the transcriptional activator will be reconstituted. Transcription of an appropriate reporter gene (e.g., Lac Z or HIS3) containing upstream GAL4 binding sites is used to indicate interaction between the two protein.

Our experiments show that the fusion proteins of full length Pre S1 protein and GAL4 DNA binding domain present transcriptional activation function in yeast. Under such a circumstance in yeast two-hybrid assay, one solution is that it may be possible to remove the transcriptional activating sequence of target protein (i.e. Pre S1 protein) by creating specific deletions of the gene and assaying the deletion constructs for those that no longer have transcriptional activation function. By assaying the three deletion constructs, the results indicate that fusion proteins of Pre S1 (12-91AA) and GAL4 DNA binding domain activate transcription in yeast in the presence of GAL4 transcription activation domain and that fusion proteins of Pre S1 (21-47AA) with GAL4 DNA binding domain have transcriptional activation function in yeast. Previous studies suggest that the hepatocyte receptor binding site is located in the 21-47 amino acids of HBV Pre S1 protein.^[1,2] So, the transcriptional activating sequence of Pre S1 protein overlaps the hepatocyte receptor binding sites, which may prevent the researchers from using yeast two-hybrid system to clone HBV receptor interacting with Pre S1 protein. However, the Pre S1 protein does not show transcriptional activation function in mammalian cells. While the two-hybrid system has been generally performed in yeast cells, it works similarly in mammalian cells (mammalian two-hybrid system).^[11,12] Our studies suggest that mammalian two-

hybrid system may be a practical method to clone HBV receptor interacting with Pre S1 protein.

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Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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