Hepatitis B virus preS1 functions as a transcriptional activation domain

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Hepatitis B virus (HBV) preS1 fused to the GAL4 DNA-binding domain functioned as a transcriptional activation domain in yeast and mammalian cells. The GAL4–preS1 fusion proteins derived from the preS1 of all three tested HBV subtypes (adr, adw and ayw) specifically activated the transcription of a lacZ or chloramphenicol acetyltransferase reporter gene linked to a GAL4-responsive promoter in transient transfection assays using yeast or HepG2 cells, respectively. Deletion analyses showed that the segments of preS1 from residues 21 to 90 and from residues 21 to 56 are sufficient and essential for the activity, respectively. Stable expression of GAL4–preS1 in Chinese hamster ovary cells also produced transactivator activity. These results suggest that preS1 fused to any DNA-binding domain of transcription factors would have transactivation potential.

Hepatitis B virus (HBV) is an enveloped DNA virus whose envelope consists of three related surface proteins called the major (S), middle (preS2–S) and large (preS1–preS2–S) proteins. HBV infection is strongly associated with the development of hepatocellular carcinoma (HCC) (reviewed in Ganem & Varmus, 1987). Epidemiological studies demonstrated an approximate 100-fold increase in the relative risk of HCC among HBV carriers compared to noncarriers (Beasley, 1988), but the precise role of HBV in the etiology of HCC is not well understood. However, the HBV X gene product and truncated preS2/S polypeptide (preS2/S) have been shown to have transactivator functions (Kekule et al., 1990; Balsano et al., 1991), and recently the integrated S gene fragment derived from human HCC was also shown to have transactivation potential (Ramesh et al., 1994). These findings strongly support the hypothesis that transactivation of cellular genes by integrated viral DNA might play a crucial role in the pathogenesis of HCC (Sherker & Marion, 1991; Okuda, 1992). In addition, integrated viral DNA undergoes extensive alterations, such as micro-deletions and translocations (Nakamura et al., 1988), and these have been suggested to be the most common changes leading to altered host gene functions. Such HBV DNA integration and aberrant host gene expression have been demonstrated for a retinoic acid receptor (de The et al., 1987), cyclin A (Wang et al., 1990) and oncogene N-ras (Déjean et al., 1986) in several human HCC cases.

<table>
<thead>
<tr>
<th>DNA construct</th>
<th>A$_{420}$ (average)</th>
<th>Relative β-galactosidase activity (%)</th>
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<tbody>
<tr>
<td>Gal4 binding domain</td>
<td>0.005</td>
<td>1 %</td>
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<tr>
<td>Gal4 binding domain preS1(119)</td>
<td>0.29</td>
<td>100 %</td>
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<tr>
<td>Gal4 binding domain preS1(90)</td>
<td>0.29</td>
<td>100 %</td>
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<tr>
<td>Gal4 binding domain preS1(56)</td>
<td>0.29</td>
<td>100 %</td>
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<tr>
<td>Gal4 binding domain preS1(119)</td>
<td>0.16</td>
<td>55 %</td>
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<tr>
<td>Gal4 binding domain preS1(57–119)</td>
<td>0.01</td>
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<td>Gal4 binding domain preS1(57–119)</td>
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<td>Gal4 binding domain preS1(119, adw)</td>
<td>0.01</td>
<td>2 %</td>
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<tr>
<td>Gal4 binding domain proS1(108, ayw)</td>
<td>0.01</td>
<td>2 %</td>
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Fig. 1. Transactivation potentials of the plasmids encoding the wild-type and various GAL4–preS1 fusion proteins in yeast cells. Most of the HBV DNA sequences were derived from pHBV315 (adr subtype; Kim & Kang, 1984) and fused downstream of the GAL4 DNA-binding domain in pGAL79 (Clontech). The average value of β-galactosidase activity in each transformant was obtained from three independent experiments with less than 15% variation. The transactivation potentials conferred by the constructs is represented by percentage conversion of the relative value to β-galactosidase activity of GAL4–preS1 (1–119).

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In this study, we have found that HBV preS1 polypeptide functions as a transcriptional activation domain when it is fused to the DNA-binding domain of transcriptional activator GAL4. All the GAL4 fusion proteins derived from the preS1 of three HBV subtypes (adr, adw and ayw) exhibited transactivator activity in both yeast and mammalian cells. In addition, we could identify specific segments of the preS1 that were either essential or sufficient for transactivation activity. These results suggest that preS1 fused to any DNA-binding domain of transcription factors, which may be generated as a consequence of HBV DNA integration and subsequent DNA rearrangement, would have transactivation potential and might play a role in the pathogenesis of HCC.

Fig. 1 depicts various plasmid constructs carrying the GAL4 DNA-binding domain with or without the preS1 gene or its segments, and their transactivator activities in yeast cells. For analysis of transactivating ability, each construct was introduced into yeast cells (trp⁻ leu⁻ Saccharomyces cerevisiae sfy526) which harbour the lacZ reporter gene linked to the promoter of the GAL1 gene with an upstream activation site sfy526) which harbour the lacZ reporter gene linked to the promoter of the GAL1 gene with an upstream activation site containing GAL4 binding sites (Gietz et al., 1992). Transcription of the lacZ gene was measured by assaying β-galactosidase produced by recombinant yeast cells, using ONPG (o-nitrophenyl-β-D-galactopyranoside) as substrate, as described previously by Miller (1972).

As shown in Fig. 1, GAL4–preS1 showed transactivator activity, while GAL4–preS2 did not, and the activity of GAL4–preS1−preS2 was the same as that of GAL4−preS1, indicating that preS1, but not preS2, can function as a transcriptional activation domain. Truncation of 30 amino acids from the C-terminus of preS1, generating GAL4−preS1(1–90), increased the activity by about 3-fold and GAL4−preS1(1–56) retained minimal activity. However, GAL4−preS1(57–119) did not show any activity. Fusion of either preS2 or any arbitrary protein domain [we used anti-HBV preS2 single-chain antibody ScFv-S2 (Park et al., 1994), which is composed of heavy and light chain variable regions linked by a peptide linker] to GAL4−preS1(1–56) increased the transactivator activity up to about 60% of GAL4−preS1 activity, whereas fusing the same protein domains to GAL4−preS1(57–119) had no effect on the activity. Taken together, these results suggest that the N-terminal half of preS1 is essential for activity and the C-terminal half may have a structural role, and that preS1(1–90) may have the optimum size and structure to mediate transactivation in yeast cells. PreS1 itself and the GAL4 DNA-binding domain, as negative controls, showed no activity. Also, the GAL4 DNA-binding domain fused to preS2/S1 or preS1/preS2/S1, which were shown to be localized in the endoplasmic reticulum and indirectly act by utilizing transcription factors NF-xB, AP-1 and AP-2 for transactivation (Meyer et al., 1992; Lauer et al., 1994), did not display any activity, suggesting that the mechanisms by which preS1 and preS2/S1 mediate transactivation are different from each other.

To examine whether the transactivator activity of GAL4–preS1 is restricted to preS1 derived from the adr subtype of HBV, the preS1 region genes of adw (Moriarty et al., 1981) and ayw subtypes were synthesized by PCR and the activities of their GAL4 fusion proteins in yeast cells were measured. The results showed that GAL4–preS1 derived from all the HBV subtypes exhibited activity, although preS1 of the adw sequence conferred lower activity compared to those of the other two subtypes (Fig. 1). Thus, the function of preS1 is not limited to adr subtype but common to all HBV.

Among eukaryotic transcriptional activators, those with acidic activation domains have been shown to function universally in eukaryotic organisms ranging from yeast to humans (Plashne, 1988). Therefore, in order to investigate whether or not the function of GAL4–preS1 is conserved in eukaryotes, the GAL4−preS1(1–90) gene was fused to the Rous sarcoma virus (RSV) promoter of mammalian expression vector pREP8 (Invitrogen) and cotransfected with the GAL4-responsive chloramphenicol acetyltransferase (CAT) reporter plasmid G5E1bCAT (Lee et al., 1990) into human hepatoma HepG2 cells using lipofectin (Gibco). The cells were then harvested 48 h after transfection in order to assay CAT activity, as described by Gorman et al. (1982). The difference in transfection efficiency was normalized by using a second reporter plasmid containing the human growth hormone gene in these cotransfection experiments. The plasmid encoding GAL4–VP16 (Fields & Jang, 1991), which is known to be the most potent transcriptional activator (Sadowski et al., 1988), was used as a positive control. The reporter plasmid contains five GAL4 binding sites upstream of the TATA box sequence of the adeno-virus E1b transcriptional unit linked to the CAT reporter gene (Fig. 2a). To begin with, we used 1, 3, 5 or 10 µg of activator plasmid and 3 µg of reporter plasmid to cotransfect cells. The results showed that GAL4–preS1(1–90) strongly transactivated the transcription of the reporter gene in HepG2 cells, irrespective of the concentration of DNA that we used (Fig. 2b), suggesting that the transactivator function of GAL4–preS1 is conserved in eukaryotes from yeast to humans.

The transactivator activity of various GAL4–preS1 derivatives was also determined in HepG2 cells (Fig. 2c). As in yeast cells, GAL4–preSl(1–90) exhibited the strongest transactivator activity, while GAL4–preS1(1–56) retained minimal activity. The GAL4–preS1(57–119), as well as negative controls such as a reporter plasmid lacking GAL4 binding sites (E1bCAT) (Lee et al., 1990) and GAL4 DNA-binding domain [GAL4 (1–147)] or preS1 itself, showed no activity. Also, all GAL4–preS1 from the three tested subtypes (ayw, adw and adw) exhibited activity, and the relative strength of the activities conferred by these three subtypes were the same as in yeast cells (Fig. 2d). These results clearly demonstrate that preS1, when fused to the GAL4 DNA-binding domain, functions as a universal transcriptional activation domain in cells, from yeast to humans, and that the N-terminal half of preS1 is essential for this function. Relative strengths of the activities of GAL4–preS1(1–90) and
GAL4–preS1 as a transcriptional activation domain

Fig. 2. Transactivator activities of various GAL4–preS1 derivatives in HepG2 cells. (a) Reporter plasmid. (b) Transactivator activity of GAL4–preS1. (c) Transactivator activities of various GAL4–preS1 derivatives. (d) Transactivator activities of GAL4–preS1 derived from the preS1 of three subtypes of HBV. (e) Transactivator activity of partially deleted GAL4–preS1(1–90).

Fig. 3. Expression and transactivator activity of GAL4–preS1(1–90) in stably transformed CHO cells. The number on the top of each lane indicates the independent cell clone producing GAL4–preS1(1–90) and lane C indicates CHO cells.

GAL4–preS1(1–56) to those of GAL4–preS1 were differential between yeast and human. This discrepancy may be due to the difference in the structure of transcription apparatus components interacting with GAL4 fusion proteins, or the stability of GAL4 fusion proteins in yeast and human cells.

In order to examine whether the segment essential for transactivator activity could be delineated, the preS1(1–90) was partially deleted and the activities of GAL4–preS1 derivatives in HepG2 cells were determined. As shown in Fig. 2(e), truncation of the N-terminal 19 amino acids, generating GAL4–preS1(20–90), did not affect activity, whereas GAL4–preS1(1–72) and GAL4–preS1(1–56) retained 10% and 7% of the GAL4–preS1(1–90) activity, respectively, but deletion of the internal region (residues 21–56) completely abolished activity. These results suggest that the preS1(20–90) is sufficient, and the preS1(21–56) is essential, for activity.

To examine whether GAL4–preS1 exhibits transactivator activity in permanently transformed cells, the GAL4–preS1(1–90) gene was placed under the control of the human cytomegalovirus (HCMV) promoter in a pRc/CMV vector (Invitrogen) containing the neomycin drug resistant marker. The resulting construct was introduced into Chinese hamster ovary (CHO) cells. After 2 weeks of selection in medium containing G418 (550 µg/ml), 11 clones were randomly isolated and transfected with the reporter plasmid G5E1bCAT for the CAT assay. As shown in Fig. 3, the permanently transformed cells did indeed transactivate transcription of the reporter gene. The difference in transactivator activity among the clones seems to reflect the different expression levels of GAL4–preS1 protein in the clones. In the control experiment, cells transformed with GAL4 plasmid did not show any transactivator activity (Fig. 3, lane C). This result again demonstrates the transactivator function of the protein. In contrast, cells transfected with plasmid DNA encoding GAL4–VP16 did not survive, most likely due to the squelching effect (Ptashne, 1988). Accordingly, it is likely that the transactivation mediated by GAL4–preS1 may involve weak interactions rather than one, or a very few, strong ones.

Amino acid sequence analysis of preS1 of the three HBV subtypes revealed that six aspartic acid residues are concentrated in the aa 21–56 segment, as found in many acidic...
activators (Ptashne, 1988), whereas no acidic residues are found in the aa 57–90 segment (data not shown). Considering that preS1(21–56) was essential for transactivator function, the acidic segment may specify the transactivation function by directly interacting with the component(s) of the transcription apparatus, and the hydrophobic region (aa 57–90) may have an important structural role or enhance the specificity of the interaction by hydrophobic force. Interestingly, preS1, but not preS2, was shown to be rich in proline residues, which constitute about 18% of the total amino acid residues, and these were scattered rather than tandemly repeated (data not shown). The exact functions of proline-rich regions are unknown. However, because of the restricted mobility of the cyclized chain (Williamson, 1994), it is conceivable that they may form a framework which enables preS1 to fold into a unique structure that can interact with the components of the transcription apparatus. Taken together with the fact that the residues from 21 to 56 contain the virus-neutralizing epitope and the hepatocyte receptor binding site (Neurath et al., 1986, 1989), this segment seems to be multifunctional.

We have confirmed that the fusion product of preS1 with the GAL4 DNA-binding domain can function as a transcriptional activation domain. Because integrated viral DNA undergoes extensive alterations (Nakamura et al., 1988), the integrated preS1 region gene may recombine with any host genes encoding transcription factors and hence give rise to novel genes encoding a functional transactivator, which might play a causative role in HBV-associated HCC. Work is in progress to obtain more definite evidence to support this hypothesis.

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References


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