Hepatitis B Virus Core Protein Interacts with the C-Terminal Region of Actin-Binding Protein

Chiu-Jung Huang  Yuan-Han Chen  Ling-Pai Ting
Institute of Microbiology and Immunology, School of Life Science, National Yang-Ming University, Shih-Pai, Taipei, Taiwan, Republic of China

Key Words
Hepatitis B virus • HBV core protein • HBV precore protein • Actin-binding protein • Chronic HBV infection

Abstract
Hepatitis B viral core protein is present in the nucleus and cytoplasm of infected hepatocytes. There is a strong correlation between the intrahepatic distribution of core protein and the viral replication state and disease activity in patients with chronic hepatitis. To understand the role of core protein in the pathogenesis of HBV, we used a yeast two-hybrid system to search for cellular proteins interacting with the carboxyl terminus of core protein, as this region is involved in a number of important functions in the viral replication cycle including RNA packaging and DNA synthesis. A cDNA encoding the extreme C-terminal region of human actin-binding protein, ABP-276/278, was identified. This interaction was further confirmed both in vitro and in vivo. In addition, the extreme C-terminal region of ABP-276/278 interacted with the nearly full-length HBV core protein. Since this region is present in both the core and the precore proteins, it is likely that both core and precore proteins of HBV can interact with the C-terminal region of ABP-276/278. The minimal region of ABP-276/278 which interacted with the HBV core protein was the C-terminal 199 amino acid residues which correspond to part of the 23rd repeat, the entire 24th repeat and the intervening hinge 11 region in ABPs. The potential functional outcome of ABP interaction in HBV replication and its contribution to the pathological changes seen in patients with chronic HBV infection are discussed.

Introduction
Human hepatitis B virus (HBV) is a small enveloped DNA virus. Acute and chronic HBV infection is a major health problem worldwide. Infectious HBV particle is a 42-nm viral particle that is composed of an outer envelope and a 28-nm inner nucleocapsid. Nucleocapsids are made up of core proteins and enclose a circular, partially double-stranded viral DNA genome. Though a DNA virus, HBV replicates through an RNA intermediate. Four open reading frames are present in the 3.2-kb HBV genome. Core protein, precore protein and a related secreted protein, e antigen, are encoded by the preC/C open reading frame [30]. The precore protein, p25, is the precursor of e antigen. Precore and core proteins are colinear except that precore protein is initiated from an upstream ATG which...
results in a 29-amino acid (aa) amino (N)-terminal extension compared with core protein. Because the first 19 aa of this segment act as a signal peptide, the precore protein is directed into the secretory pathway. This 19-aa segment is subsequently removed to generate a p22 protein intermediate that is either translocated across the endoplasmic reticulum membrane or released back into the cytosol and then transported into the nucleus [29]. In the posttranslational reticulum, most likely the Golgi apparatus, p22 is cleaved at the carboxyl (C) terminus in the arginine-rich domain to generate soluble e antigen that will be secreted from the cell [9, 28, 37]. The predominant cleavage site is at aa 149 [26]. Core protein, however, retains this arginine-rich domain.

Core protein forms dimers which then assemble into nucleocapsids. Packaged inside of nucleocapsids are polymerase and pregenomic RNA which serves as the template for DNA synthesis. The N-terminal 144-aa segment of core protein is essential to direct the assembly of nucleocapsids [8, 12]. The protamine-like C terminus, residues from aa 150 to 185 (adw) or 183 (ayw), is dispensable for capsid assembly but mediates interaction between core protein and nucleic acid. This region, which plays an important role in viral DNA synthesis and genome packaging, contains four blocks of arginine residues and three overlapping SPRRR [12, 25, 45].

Core protein can be found in the nucleus and cytoplasm of infected hepatocytes [1, 13]. The expression and nuclear transport of core protein are tightly regulated. The first and the fourth blocks of arginine residues constitute the nuclear localization signal [6, 42]. The core protein is also a phosphoprotein. The phosphorylation sites in core protein are the serine residues in the SPRRR motifs located at the C terminus [21, 43]. The nuclear localization of core protein is increased during the G1 phase, reduced to an undetectable level during the S phase, and increased again when cells are arrested in the G0/G1 phase. The phosphorylation status of core protein correlates with its intracellular localization. The serine-to-alanine substitutions of core protein enhance its nuclear localization. These results suggest that phosphorylation may negatively regulate the nuclear localization of core protein [18, 44]. A recent study indicated that both Ser164 and Ser172 were important for pregenomic RNA packaging and viral replication, whereas Ser157 was not essential for pregenomic RNA packaging, viral replication, or viral maturation [15]. These findings provide indirect evidence that important events in HBV infection are regulated by the phosphorylation status of core protein.

There was a strong correlation among the intrahepatic distribution of core protein, the viral replication state, and disease activity in chronic hepatitis. In e-Ag-positive patients with minor hepatitis activity, core protein was distributed mainly in the nuclei. In e-Ag-positive patients with chronic active liver disease, the nuclear expression of core protein decreased significantly with an increase, instead, in cytoplasmic/membranous core protein expression. In carriers who are anti-e positive, core protein is undetectable anywhere. These clinicopathologic examinations indicate that cytoplasmic/membranous expression of core protein correlates with an elevated degree of inflammation in liver cells [1, 13]. Core protein expressed in given temporal-spatial patterns may contribute to the cytopathologic changes seen in patients with persistent HBV infection.

The close association of core protein expression with disease activity in patients with chronic HBV infection suggests that core protein may play causative roles in chronic liver damage. We therefore set out to identify the cellular protein(s) that interact with core protein by yeast two-hybrid screening. We choose to focus on the C-terminal portion of core protein first as this region is phosphorylated and the phosphorylation status of core protein correlates with intracellular localization. This portion of core protein may also interact with cellular protein(s) in facilitating genome packaging and/or replication. A cDNA encoding the C-terminal region of cellular actin-binding protein (ABP) was identified.

Materials and Methods

Plasmid Constructions

The HBV sequence used in the study was of the adw subtype. The plasmid pLexA-2/5C contained the HBV core protein from aa 119 to 185, designated as 2/5C, which was fused in a frame downstream of the LexA DNA-binding domain of a pLexA vector. pLexA-2/5C was used as a bait in the yeast two-hybrid screen, pLexA-2/5Cd1/5C was identical to pLexA-2/5C except that it lacked the SPRRR motifs and contained the core sequence from aa 122 to 145. pLexA-BD and pLexA-Lam, respectively, encoded a LexA DNA-binding domain protein and a LexA-lamin C fusion protein which served as negative controls.

The plasmid pGST-2/5C was used to express the recombinant glutathione S transferase (GST)-2/5C fusion protein in Escherichia coli as previously described [14]. GST-2/5C contained the same core sequence as in pLexA-2/5C.

For expression in eukaryotic cells, GST and different GST-core fusion proteins were driven by the cytomegalovirus (CMV) immediate-early promoter present in the plasmid pCMV. Plasmid pCMVGST contained a parental GST open reading frame derived from the pGEX-3X vector. GST-2/5C in pCMVGST-2/5C contained