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Fluorescent protein tagged hepatitis B virus capsid protein with long glycine-serine linker that supports nucleocapsid formation

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ABSTRACT

Fusion core proteins of Hepatitis B virus can be used to study core protein functions or capsid trafficking. A problem in constructing fusion core proteins is functional impairment of the individual domains in these fusion proteins, might due to structural interference. We reported a method to construct fusion proteins of Hepatitis B virus core protein (HBc) in which the functions of fused domains were partially kept. This method follows two principles: (1) fuse heterogeneous proteins at the N terminus of HBc; (2) use long Glycine-serine linkers between the two domains. Using EGFP and RFP as examples, we showed that long flexible G_4S linkers can effectively separate the two domains in function. Among these fusion proteins constructed, GFP-G_4S186-HBc and RFP-G4S47-HBc showed the best efficiency in rescuing the replication of an HBV replicon deficient in the core protein expression, though both of the two fusion proteins failed to support the formation of the relaxed circular DNA. These fluorescent protein-tagged HBcs might help study related to HBc or capsids tracking in cells.

1. Introduction

Hepatitis B virus (HBV) is an enveloped virus which packages its DNA genome in an inner icosahedra capsid. The capsids are built with core proteins (HBc), which are 183–185 amino acid residues long. According to the function, HBc can be divided into two domains: the assembly domain (amino acid residues 1–140, Fig. 1A) which is necessary and sufficient to form dimers, and the carboxy-terminal domain (CTD) (residues 150–183, Fig. 1A) which has a high affinity for RNA(Porterfield et al., 2010) and is needed for pgRNA encapsidation (Nassal, 1992). By providing a functional room for genome DNA replication, the capsids apparently are indispensable for the fulfillment of HBV life cycle (for reviews, see (Seeger and Mason, 2000, Nassal and Schaller, 1993, Seeger and Mason, 2015)).

Structure of HBV capsid has been studied extensively by both cryo-EM and X-ray crystallography (Bottcher et al., 1997, Crowther et al., 1994, Wynne et al., 1999, Dryden et al., 2006, Conway et al., 1997). Bacterially expressed core proteins spontaneously assemble into two types of capsids, resembling the native capsids morphologically(Dryden et al., 2006), composed of 180 or 240 core proteins arranged with T = 3 or T = 4 icosahedral symmetry, respectively (Bottcher et al., 1997, Conway et al., 1997, Crowther et al., 1994, Wynne et al., 1999). The dimeric spikes formed by 4-helix bundles protrude from the surface of capsids. At the tips of the spikes are the major immunodominant region (MIR) of the HBc molecule presented by amino acids 78–82 (Bottcher et al., 1997, Wynne et al., 1999; Fig. 1A). The two N termini per dimer were located on either side of the spike-stem, at the level at which it enters the shell (Conway et al., 1998), and the C termini lines the capsid interior (Zlotnick et al., 1997; Wang et al., 2012; Fig. 1A).

Fusion core proteins have been exploited to study core protein functions. Green fluorescent protein (GFP) is usually fused with HBc to facilitate observation. Schaller's lab used an N-terminally fused GFP-HBc to investigate the factors influencing the intracellular distribution of HBc (Weigand et al., 2010). The same lab used a line of N-terminally fused GFP-DHBcs to identify the nuclear localization signal that mediates nuclear pore association of the duck hepatitis B virus nucleocapsid (Mabit et al., 2001). A COOH-terminally truncated core protein with GFP inserted into the immunodominant loop (amino acids 78–83)

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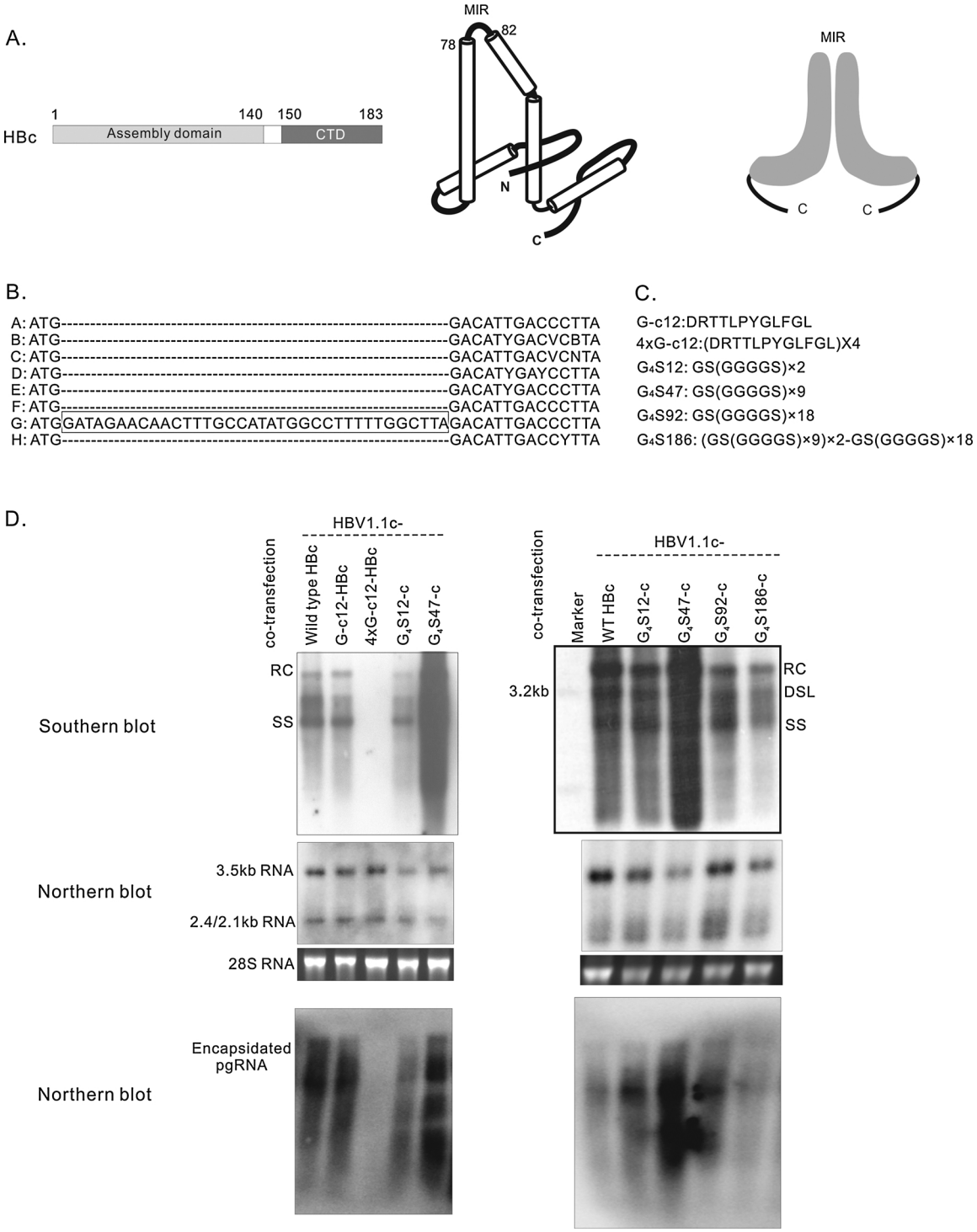

Fig. 1. The N terminus of core protein tolerates modification. (A) Structure models of HBc monomer and dimer. (B) Alignment of the start part of the ORFs of HBc gene of A to H genotype. The sequences are from the whole genome of 831 HBV strains retrieved from Genebank (Hu et al., 2009). There is an extra 12aa insertion at the N terminus of HBc of genotype G. (C) Amino acid sequences added at the N terminus of genotype D HBc. (D) Functional assay of core fusion proteins. Plasmids expressing wild type or engineered core proteins were co-transfected with HBV1.1c^{-} into HepG2 cells respectively. Intracellular core DNA was extracted and detected by Southern blot. Total RNA or encapsidated RNA was extracted and detected by Northern blot. RC, relaxed circular DNA; DSL, double-strand linear DNA; SS, single-strand DNA. pgRNA, pregenomic RNA.

(Kratz et al., 1999) has been used to investigate the interaction of hepatitis B virus capsids with nuclear pore complexes in permeabilized HeLa cells (Lill et al., 2006).

The above studies using GFP-HBc fusion proteins provided much helpful information about the biological features of HBc. However, whether these fusion proteins keep the authentic functions of HBc is not so clear. One fact is that the N-terminally fused GFP-HBc is unable to form capsids (Weigand et al., 2010). Although the core protein with GFP inserted into the immunodominant loop did allow capsids formation (Kratz et al., 1999, Lill et al., 2006), our results showed that this fusion protein did not support HBV DNA replication in HepG2 cells (Chen et al., 2013). These data indicated that the functions of HBc were not fully kept in these GFP-HBcs, might attributable to the structural interference between the two proteins fused together. A core fusion