Complementary roles of the S and L envelope proteins in the capsid recruitment and the HBV budding

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AIM OF THE STUDY

Hepatitis B virus (HBV) morphogenesis involves an interaction between envelope proteins and the mature nucleocapsid that results from an auto-assembly of the core protein and the newly synthetized pregenomic RNA (pgRNA). Although theses mechanisms are still poorly characterized, the observation that most of HBV particles from infected patients remain highly infectious suggests that HBV morphogenesis and secretion is a very efficient and closely regulated process. This reinforce the hypothesis that only assembled capsids which have undergone a maturation step are compatible for the acquisition of the viral envelope, thus allowing membrane binding and subsequent budding. Maturation of the capsid occurs gradually with the synthesis of the definitive HBV DNA genome (RC-DNA). This viral DNA synthesis is thought to induce subtle structural modifications of the assembled nucleocapsid, allowing its interaction with envelope proteins.

In this study, our goal was to determine how the mature nucleocapsid is specifically recruited for envelopment. To do so, we developed *in vitro* cellular models allowing us to compare the ability of empty assembled HBV capsid vs mature nucleocapsid to differentially interact with the S and L HBV envelope proteins.

DESIGN AND EXPRESSION OF HBV ENVELOPE PROTEINS MUTANTS

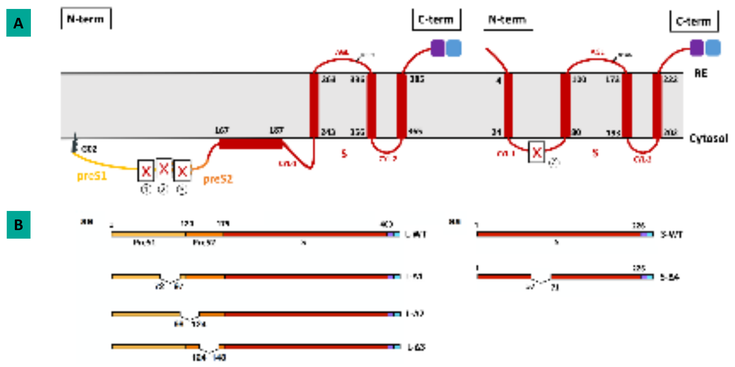


Figure 1 : Description of envelope proteins mutants. (A) Envelope protein mutants are presented in their ipreS topology. (B) Deletion mutants of L comprise L-**Δ1** (72 – 97), L-**Δ2** (98 - 124) and L-**Δ3** (72 – 97) around the preS1-preS2 region. One S mutant was designed with a deletion in the first cytoplasmic loop ; S-**Δ4** (57 – 71). All mutants were elaborated with HA- and His-tag (in purple and blue respectively) located at the C-terminal end of the proteins. Design of the mutants was based on the following publications : **Poisson F. (1997), Bruss V. (1997), Löffler-Mary H. (2000).**

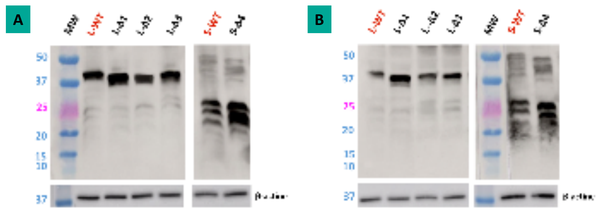


Figure 2 : Detection of the mutants by western-blotting experiments. *Wild type* L and S proteins are in red and the mutants in black. (A) Western-blot incubated with a monoclonal anti-HBs antibody (Fitzgerald). (B) Western-blot incubated with a polyclonal anti-His antibody (Sigma). β-actin detected by a polyclonal antibody (abcam) is used for the normalisation.

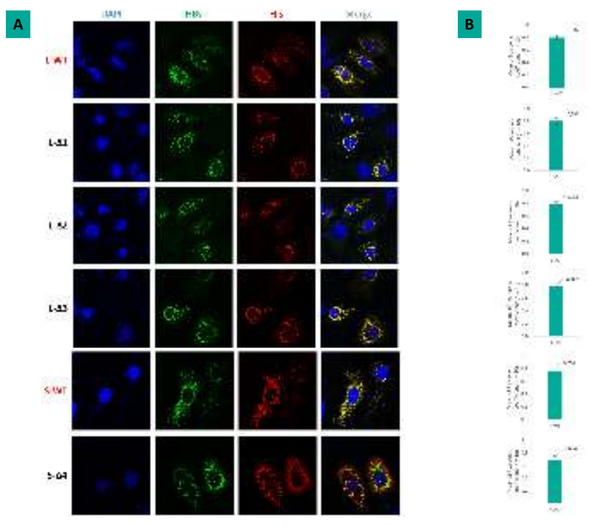
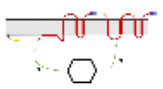


Figure 3 : Detection of the mutants by confocal microscopy. (A) Nucleus in blue are labelled with DAPI. Envelope proteins in green are labelled with an anti-HBs antibody (Fitzgerald). His-tag proteins in red are labelled with an anti-His antibody (Sigma). (B) Pearson's coefficient. Ten photographies per conditions were used to calculate the colocalisation level.

All mutants are expressed at a similar level to the *wild type* envelope proteins L and S, respectively. Moreover, we observed a close identical sub-cellular localisation of both *wild type* and mutated envelope proteins, regardless of the antibody used for detection (anti-HBs and anti-His tag).

NON REPLICATIVE CONTEXT

Wild type envelope proteins were co-expressed with the core protein in **Huh-7 cells in absence of viral replication**. After 3 d.p.t, cells were harvested and subjected to confocal microscopy (**Fig. 4A**) and immuno-precipitation (**Fig. 4B**). Envelope protein mutants were expressed and analysed in a similar manner (**Fig. 5A,B**).



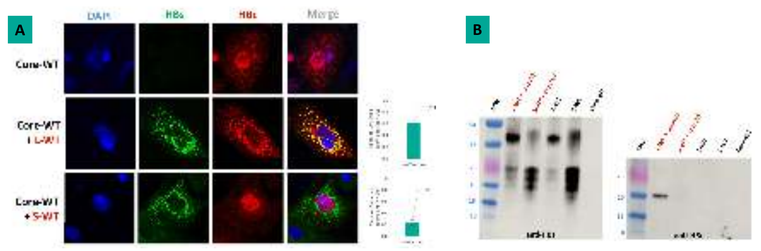


Figure 4 : Colocalisation and interaction between the core protein and *wild type* envelope proteins. (A) Nucleus in blue are labelled with DAPI, envelope proteins in green are labelled with a polyclonal anti-His antibody (Sigma) and core proteins in red are labelled with a polyclonal anti-HBc antibody (serum). The colocalisation level is determined by the Pearson's coefficient. (B) Western anti-HBs (left panel, IP) and anti-HBc (right panel, co-IP) after immunoprecipitation with an anti-His antibody (Sigma).

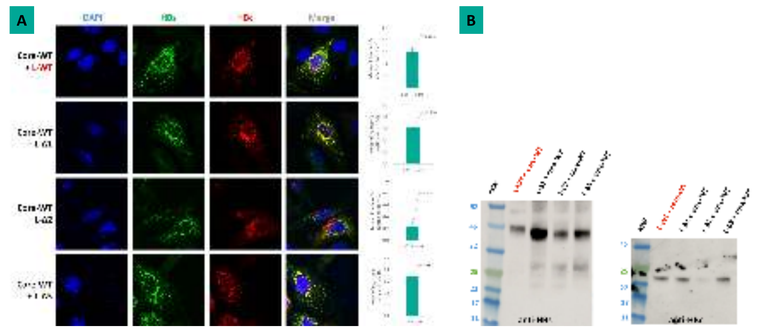


Figure 5 : Colocalisation and interaction between the core protein and envelope protein mutants. (A) Same method used in Fig. 4A. (B) Same method used in Fig. 4B.

We also used a core protein deleted for the α5 helice, a mutant known to be inefficient to self assemble into capsids. Our objectives were to confirm that the recruitment by the envelope proteins is a mechanism dependent on the capsid formation. Confocal microscopy (**Fig. 6A**) and immuno-precipitation (**Fig. 6B**) are made after co-expression of the two partners.

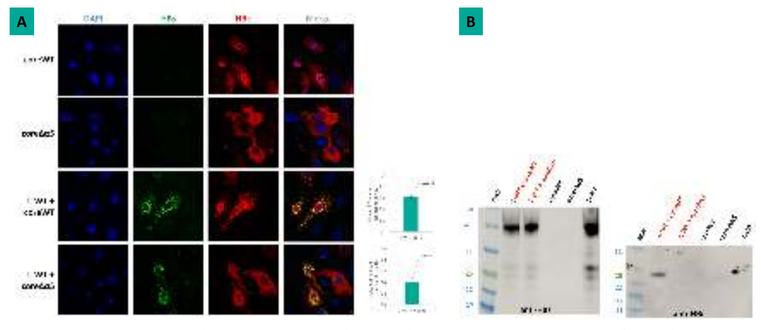


Figure 6 : Colocalisation and interaction between the core protein (*wild type* and deleted α5 mutant) and *wild type* envelope proteins. (A) Same method used in Fig. 4A. (B) Same method used in Fig. 4B.

In non-replicating cells, we show that only the L envelope protein is able to interact with the core protein using its domain covered by aa 98 to 124. On the other hand, we didn't found any interaction between the core protein and the S envelope protein, suggesting that this later protein is not compatible for an interaction with empty assembled capsids.

REPLICATIVE CONTEXT

Wild type envelope proteins were co-expressed with the core protein in **Huh-7 virus-replicating cells**. After 3 d.p.t., cells were harvested and subjected to confocal microscopy (**Fig. 7A**) and immunoprecipitation (**Fig. 7B**) experiments. Envelope protein mutants were processed in a similar manner (**Fig. 8A,B**).

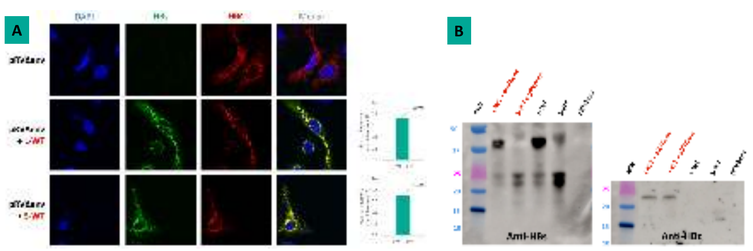


Figure 7 : Colocalisation and interaction between the core protein and *wild type* envelope proteins in cells replicating the virus. (A) Same method used in Fig. 4A. The replicative context was created thanks to the use of a plasmid which code for the HBV genome silenced for the envelope protein production : pRVHBV1.5Δenv ("pRVΔenv", **Bruss V.**). (B) Same method used in Fig. 4B.

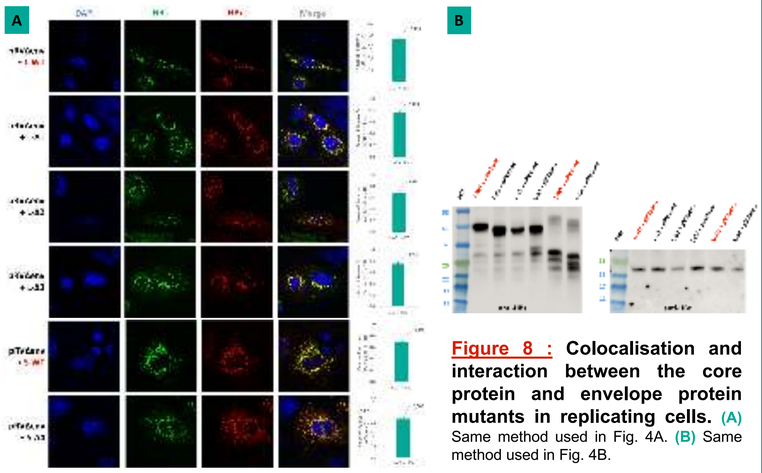


Figure 8 : Colocalisation and interaction between the core protein and envelope protein mutants in replicating cells. (A) Same method used in Fig. 4A. (B) Same method used in Fig. 4B.

In HBV-replicating cells, we show that both L and S proteins are able to interact with the core protein. Since the two envelope proteins are required for viral budding, these results suggest that 1) the L protein is dedicated to the recruitment of the assembled nucleocapsid, 2) the S protein is able to select for budding only the nucleocapsids which have undergone a maturation process. Surprisingly, we detect a weak affinity between the core and envelope mutant proteins L-Δ2 and S-Δ4. This latest interaction is currently under investigations.

PROSPECTS

The difference observed between results obtained in non replicative cells and replicative cells could be explained by the **nucleic acid content** leading to capsid maturation process.

We now have to precise how the nucleic acids content may impact viral morphogenesis. These approaches are currently under investigation using the following options :

- Expression of a **modified HBV genome defective for an active polymerase**
- *In vitro* treatment of infected cells with **entecavir** (an antiviral which block pgRNA to rc-DNA conversion).

CONCLUSIONS

Our results showed that the S and L proteins present **different affinity profiles next to the recruitment of the core protein**. When the L protein is produced alone, co-localization and co-immunoprecipitation analysis suggest that the L protein recruits the core protein independently from the replication cycle. In sharp contrast, the S protein was shown to interact with the core protein only in a replicative context. Together, these results suggest that the L protein could be a central component during viral morphogenesis , i) allowing the capsid recruitment close to membranes involved in the budding, ii) interacting with the S protein, the well-known effector of the budding. Moreover, our results suggest that the S protein could play a role as a sensor protein leading to the strict selection of mature capsids for envelopment.

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