Identification of the Hsp40 chaperone DNAJB12 in the functional mechanism of the nucleic acid polymer REP 2139

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Abstract# 1224

Lena Angelo[1], Richard Boulon[1], Matthieu Blanchet[1,2], Andrew Vaillant[2], Patrick Labonté[1]

1.Institut National de la Recherche Scientifique – Institut Armand Frappier, Laval, Canada, H7V 1B7.
2.Replicor Inc. Montréal, Canada, H4P 2R2.

INRS Institut national de la recherche scientifique · replicor · NSERC CRSNG

ABSTRACT

During hepatitis B viral (HBV) infection, subviral particles (SVP) are produced in large excess over virions and constitute the vast majority of synthesized and secreted HBs antigen (HBsAg). HBsAg is postulated to be important in allowing HBV to chronically persist by interfering with immune function. Nucleic acid polymers (NAPs) interfere with the assembly/secretion of HBV SVP without affecting the release of HBeAg or Dane particles (1). Combination therapy with the NAP REP 2139, pegylated interferon and tenofovir disopropxil fumarate leads to high rates of HBsAg loss and functional cure of HBV and HDV. Ongoing experimental verification of the molecular mechanism of NAPs driving inhibition of SVP assembly and secretion are presented.

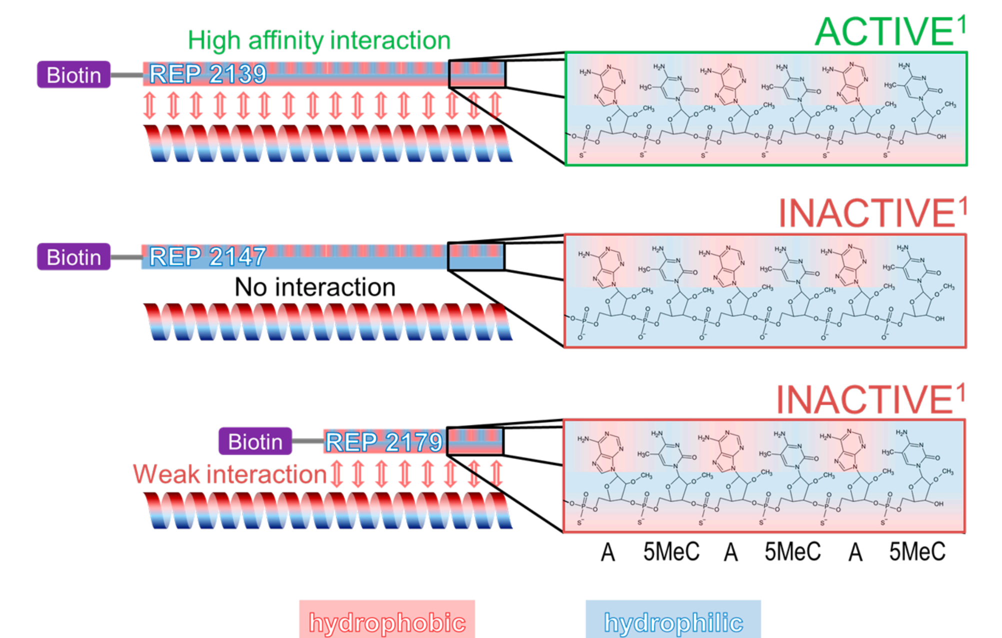
MS/MS interactome analysis identified the Hsp40 J-protein DNAJB12 as NAP interactor conforming to the known structure activity relationship for NAP antiviral activity. Silencing of DNAJB12 by shRNA knock-down experiments showed a selective effect on viral protein secretion, inhibiting up to 90% of HBsAg secretion inhibition with no inhibition of HBeAg secretion, consistent with the *in vitro* REP 2139 effect (1). Using co-immunoprecipitation of biotinylated REP 2139, DNAJB12 was readily detected in the pull-down extract confirming interaction between REP 2139 and DNAJB12.

The inhibition of SVP assembly and secretion involves the interaction of NAPs with DNAJB12. The normal function of DNAJB12 is to assist Hsc70/Hsp70 in the folding of transmembrane proteins. Indeed, DNAJB12 is located at the endoplasmic reticulum membrane where its function determines the fate of nascent transmembrane proteins which in turn may lead to the degradation of misfolded proteins. We hypothesize that the interaction of REP 2139 with DNAJB12, prevents DNAJB12 from folding nascent HBsAg and / or allowing its assembly into SVP, leading to its proteasomal/lysosomal degradation.

METHODS

NAP target identification

A differential-interactome screen of HepG2.2.15 lysate used biotinylated NAPs which bracketed the size and phosphorothioation (PS) dependent structure activity relationship of NAPs. These NAPs included the clinically active 40mer PS REP 2139 and its inactive analogs: the 40mer phosphodiester REP 2147 and the short PS (20mer) REP 2179. MS/MS analysis (three experiments per NAP) identified NAP-bound proteins at pH 7.4. DNA/RNA binding proteins or proteins with interaction selectivity ratio < 2 were excluded. Selected candidates had the greatest significant ($p < 0.05$) selective interaction ratio between REP 2139 / REP 2147 (PS-dependent) and REP 2139 / REP 2179 (size-dependent) (figure 1).



Target knock-downs (KD)

Candidates were validated by shRNA-mediated knockdown effects on HBsAg and HBeAg secretion. Efficacy of shRNA mediated mRNA knockdown was verified by RT-qPCR and Western-blot for DNAJB12 (Figure 3). Effects on the KD on HBsAg secretion were measured by ELISA (GS EIA 3.0, Biorad) and expressed as relative units (RU) normalized to total cellular protein (as determined by BCA assay).

Co-immunoprecipitation / Pull-down assays of REP 2139

We have conducted a MS/MS analysis data identified DNAJB12 as the potential target for the REP 2139 (3). In order to validate this interaction, co-immunoprecipitation and pull-down assays were performed on biotinylated REP 2139 (REP 2139-B). HepG2.2.15 cells were lysed with NP40 lysis buffer and incubated overnight with 50 nM of either REP 2139 or REP 2139-B. Magnetic protein G beads were coupled overnight with anti-biotin antibody. After several washes, HepG2.2.15 lysate incubated with NAPs were incubated with either the protein G bead coupled with anti-biotin (co-immunoprecipitation) or with streptavidin beads (pull-down). The supernatants (S) of the beads were kept (figure 4) and both the supernatant and eluates (IP) were analyzed by western blot.

RESULTS

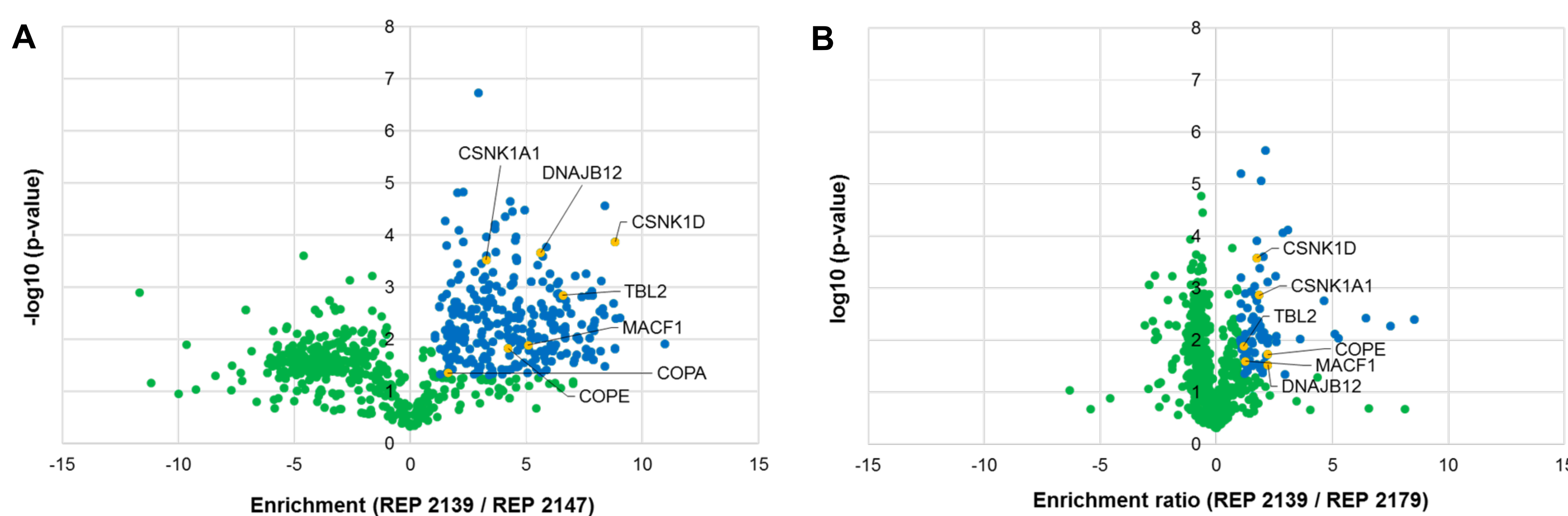


Figure 1. MS/MS identification of NAP interactors
Volcano plots derived from MS/MS interaction analysis for hydrophobic selective (A) and size selective (B) interaction of proteins at pH 7.4 from HepG2.2.15 cells with NAPs. No interactions with HBV proteins were observed. Candidates with the greatest hydrophobic and size selective interactions are indicated.

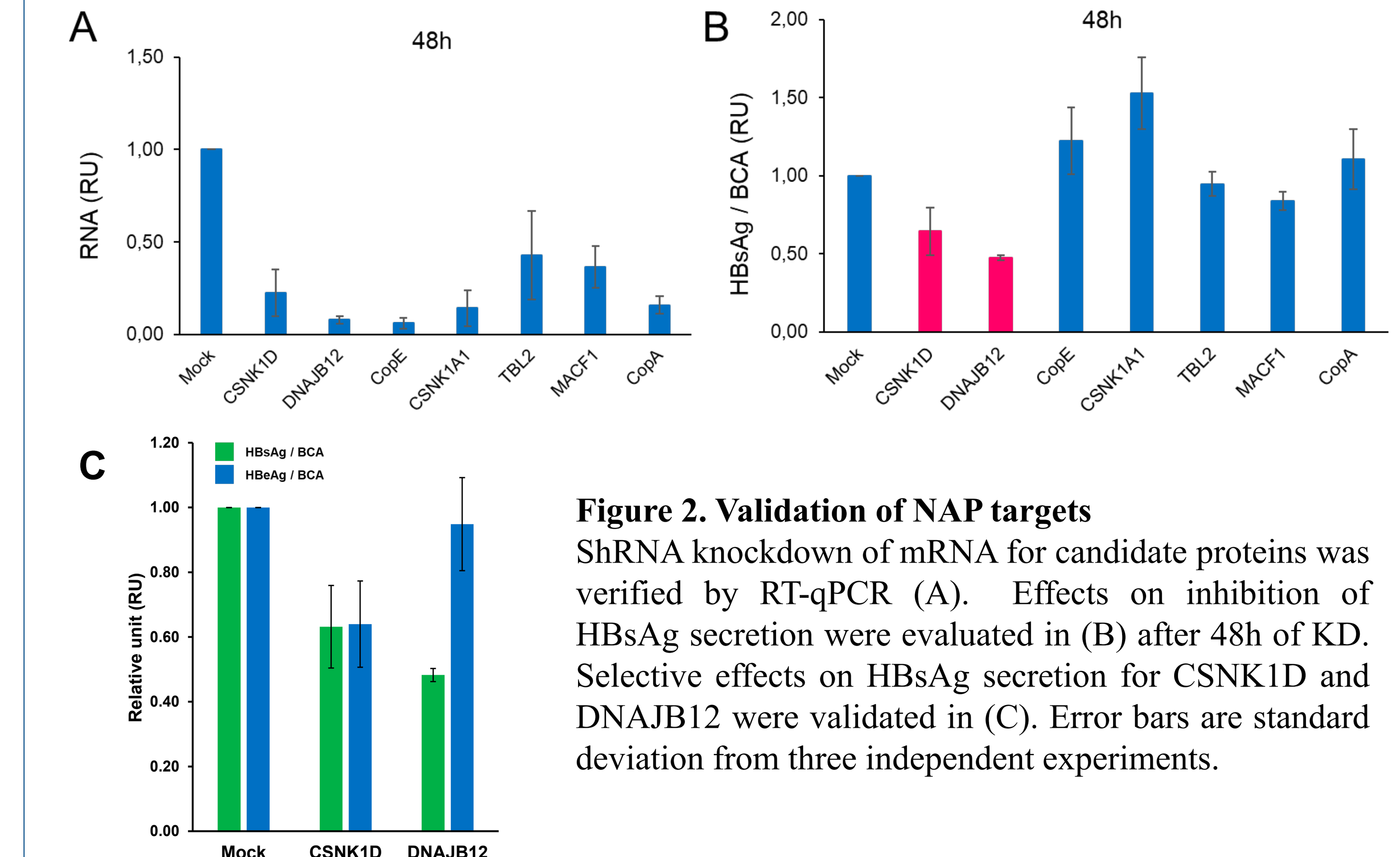


Figure 2. Validation of NAP targets
ShRNA knockdown of mRNA for candidate proteins was verified by RT-qPCR (A). Effects on inhibition of HBsAg secretion were evaluated in (B) after 48h of KD. Selective effects on HBsAg secretion for CSNK1D and DNAJB12 were validated in (C). Error bars are standard deviation from three independent experiments.

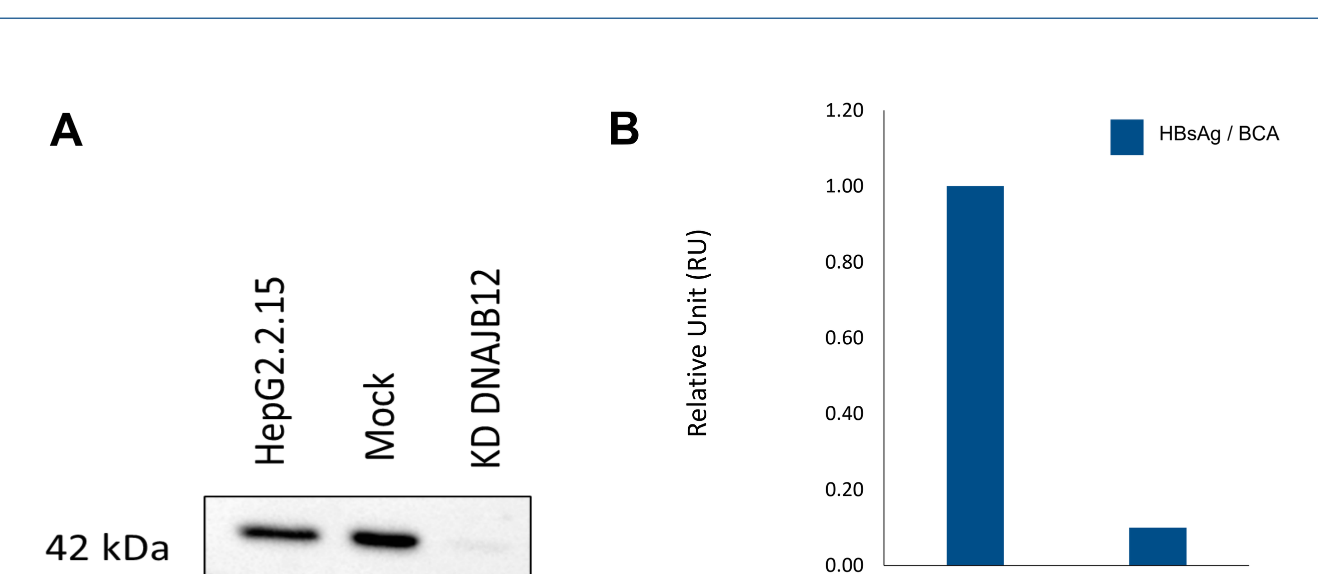
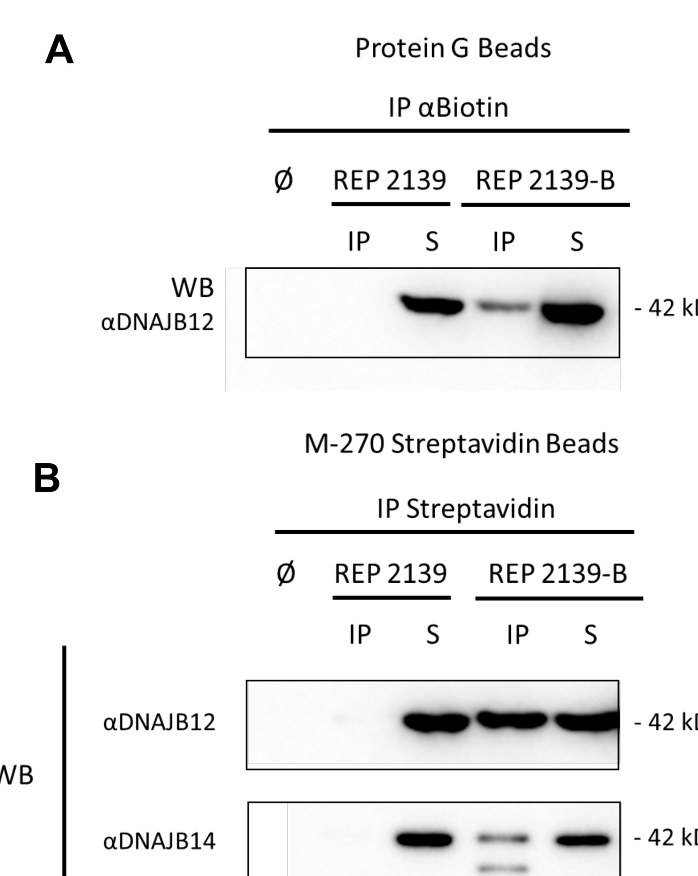


Figure 3. DNAJB12 Knock-down and effects on HBsAg secretion.
(A) Western-blot for DNAJB12. DNAJB12 level was determined in HepG2.2.15 cells, in shRNA negative cell line (mock) or in knockdown DNAJB12 cell line (KD DNAJB12) at day 6.
(B) Effects on inhibition of HBsAg secretion 6 days post-transfection of DNAJB12 shRNA lentivirus.

Figure 4. Interaction of REP 2139 with DNAJB12
(A) HepG2.2.15 cells were lysed and incubated with Protein G beads coupled with anti-biotin antibody and REP 2139 +/- biotin.
(B) HepG2.2.15 cells were lysed and incubated with M-270 streptavidin beads and REP 2139 +/- biotin.
For both experiment, DNAJB12 was detected in the eluate (IP) and in the remaining supernatant (S). DNAJB14 was also pull-downed with the DNAJB12 - REP 2139-B complex.



CONCLUSIONS

- **DNAJB12, a Hsp40 chaperone, is crucial for the HBsAg secretion and is targeted by REP 2139.**
- **Absence of effect on HBeAg secretion in cells knock-down for DNAJB12 is consistent with the effect of REP 2139 observed in vitro and in clinical trials.**
- **The interaction with and inhibition of DNAJB12-mediated chaperone function is consistent with the selective effect of NAPs to inhibit the assembly/secretion of spherical SVP.**

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CONTACT

Dr. Patrick Labonté patrick.labonte@inrs.ca

Dr. Andrew Vaillant availlant@replicor.com